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Malaria Mosquito Bionomic And Local Plant Extract Bioprospecting As Botanical Insecticyde In Southeast Minahasa

Rudi A. Repi[1], Yermia S. Mokosuli[2], Rantje L. Worang[3], Verawati I. Y. Roring[3]

1. Research Institution, Manado State University. Indonesia.
2. Center of Biopharmaca and Biomollecular Study, Research Institution, Manado State University
3. Department of Biology FMIPA Manado State University

* E-mail of the corresponding author: rudiarepi@mail.unima.ac.id

Abstract

A research that aims to find bionomic data and mosquito diversity of *Anopheles sp* in Southeast Minahasa and local plant extract bioprospecting as botanical insecticide has been conducted. The research result shows that there are eight species of *Anopheles spp* that are found in Lobu and Silian Village, Toluaan Sub-district, Southeast Minahasa. The total of catched mosquito as follows: *An. Barbirostris* is 1779, *An. Vagus* is 1401, *An. Subpictus* is 574, *An. Indifinitus* is 528, *An. Tesselatus* is 415, *An. Kochi* is 94, *An.* Pediataeniatus is 30 and *An. Aconitus* is 16. There are 4 species with the highest abundance in Lobu and Silian Village, i.e. *An. Barbirostris, An. Vagus, An. Subpictus* and *An. Infinitus*. The activity treatment of purple lemongrass oil (*cymbopogon citratus*) and Tembelek (*Lantana camara*) are effective as Southeast Minahasa ethnobotany plant extract anti mosquito in North Sulawesi. There is a plant extract photochemistry class content and plant extract combination. The active substance content especially in purple lemongrass oil (**α**-D-Galaktopiranosid) affects much in controlling the activity of anti-mosquito *anopheles sp.*

Keywords: bionomic, Anopeheles, bioprospection, botanical insecticyde.

1. Introduction

More than 40% of the world population (> 2.1 billion people) is estimated at risk of contracting malaria and mainly located in tropical countries which are generally developing countries (WHO, 2003). Approximately 1.2 billion people or about 85% of the total population in South Asia are at risk of malaria (Whosea, 2004). Climate pattern changes, poor sanitation, improper use of insecticides lead to the mosquito population growth as a malaria vector in various regions of the Tropics, including Indonesia. Malaria is caused by Plasmodium sp. which is transmitted to humans through the bite of Anopheles sp. Malaria transmission is influenced by several factors, i.e. parasite (plasmodium) factor, human (host) factors, Anopheles mosquito (vector) factor, and environmental factors. Anopheles mosquito is one of the malaria vectors that widespread in almost all provinces in Indonesia.

Malaria is a public health problem that is still a priority of health programs in Indonesia. Malaria can cause death for infants, toddlers, pregnant women and adults. Many malaria cases occur outside the metropolitan areas or cities that spread evenly throughout Indonesia. As tropical country, Indonesia has many malaria endemic areas that are ranging from the west to the east.

A research that is conducted suggests that virus which causes Cikungunya can be also transmitted by *Anopheles spp* mosquito as Malaria vector. In addition, dengue fever cases begin to be found in mountainous areas such as Tomohon and Langowan that are not the habitat of *Aedes aegypti* as dengue fever vector mosquito. There is strong indication that the dengue fever can be transmitted by *Anopheles spp* which is in mountainous areas. This mosquito behavior change (bionomic) is also affected by global climate change and theoretically an evolution form of a species on natural selection process.

Mosquito as disease vector is a starting point of disease spreading so that it needs to be overcome. Treatment after suffering malaria is more at risk than the precaution that is malaria mosquito population handling in domestic area. Malaria treatment in Indonesia using chloroquine has led to resistance since 1973; further, sulfadoxine pyrimethamine (SP) was also reported to have led to resistance on *Plasmodium* so it is not effective to be used anymore.

From many research reports, vector control is still better than post malaria suffering treatment. The prevention effort of malaria vector is conducted using various methods, e.g. chemistry methods, mechanics methods, and

biological control. Chemistry insecticides utilization can trigger mosquito resistance and genetic change. The research which is conducted in Africa and America in 2010 found that *Anopheles* is mosquito species which is very fast resistant and perform genetic modification (Anonymous, 2011). Thus the use of chemical insecticides in the long term can trigger the emergence of new mosquito strains which is more adaptive and can even be vectors of dangerous diseases.

Distribution of malaria endemic areas by the Department of Health put the Minahasa and Bolaang Bolaang as a malaria endemic area in North Sulawesi. Specifically in Minahasa area, Southeast Minahasa district is an area with high malaria incidence cases. In Bolaang Mongondow, Dumoga which is marshy area has the highest clinical malaria incidential case. From various research reports, both areas have high malaria vector population intensity and density. There are a lot of mosquito breeding areas with stagnant water form all year so that the continuation of the mosquito population is relatively stable almost throughout the year.

In Indonesia, there are 14 species of *Anopheles.* At the malaria vector distribution map in North Sulawesi, there are 3 species, i.e. *Anopheles barbirostris, Anopheles subpictus,* and *Anopheles parangensis* (RI Department of Health). From the data from North Sulawesi Provincial Health Department, the malaria prevalence in Southeast Minahasa is high. More than 35 Malaria cases are found that perform treatment in Hospital in 2009 and 37 cases in 2010. Data bank of RI Departement of Health in 2011 released that malaria incident on a thousand people in Southeast Minahasa is 32.27% and the number of dengue fever cases is 11 cases per a thousand people. It indicates that malaria is still a problem in Southeast Minahasa.

Malaria and dengue vector mosquito control programs in various countries, including Indonesia, is generally less successful, because almost totally dependent on the fogging to kill adult mosquitoes. It needs huge cost (5 billion per year) (Baskoro *et. al*. 2007); cause vector resistance due to improper dosage, and do not affect in long term because the mosquito larvae are not dead. *Ae aegypti* resistance on organophosphates in Salatiga ranged from 16.6 to 33.3 percent, while on malathion 0.8% reaches to 66-82 percent (Boewono *et. al*. 2006). A research in Bandung shows that *Ae aegypti* is also resistant also resistant to d-Allethrin, Permethrin and Cypermethrin with 90% Lethal time (LT90) ranged from 9-43 hours (Astari, 2005).

The insecticides use in eradicating malaria vector mosquito in Indonesia had started since 1952, i.e. the use of DDT and dieldrin in Jawa. Until 1959 it has found resistance case of *An. Aconitus* on Dieldrin and *An. sundaaicus* on DDT. Central Jawa and Yogyakarta are also malaria endemic areas in Indonesia. Malaria vector control is conducted in chemical IRS (*Indoor residul spraying*) chemical method using organophosphate and carbamate insecticides and is prioritized in HCI (*high case incidence)* areas. After discontinuation of DDT use, the alternative insecticide that is used for malaria vector control is fenitrithion 40 WP. Since 1989/1990 this insecticide has been used in Central Jawa, DIY and East Jawa. In 1991 80 WP insecticide bendiocarb also began to be used. The continuous use of insecticide in a long time and high frequency can lead to reducing the vulnerability of the target mosquitoes. The success of insecticide use higly depends on vector susceptibility to the used insecticides (Astari, 2005).

One of approaching that can be used is utilizing plant bioactive as botanical insecticide. In nature, there is balance principle that is continuously maintained through the interaction of various ecological components. As an example, in malaria endemic area, there are vaiours kinds of plant which are used as a source of raw material for making insecticides by the local community to defend themselves from malaria. This indicator is proven in Papua that many Papuans who are not infected malaria because using the plant substance that is formulated and applied throughout the body to prevent mosquito bites. This local wisdom is worth to be dug through research in order to reduce and prevent malaria.

Plant produces certain compounds as a form of self-defense from microbial attack or multicellular organisms, especially insects which become pests. Some plants that contain bioactive compounds are Acer rubrum L. (Aceraceae), Betula allenghaniensis Britton (Betulacea), Betula papyrifera Britton, K. cordiformis Caryaa Prunus serotina Ehrh (Rosaceae) etc. (Omar S, 2000). As a Malaria endemic area, Minahasa community has long been known and used certain types of plants as botanical insecticides. In order to cope the mosquito problem, Minahasa people have use pangi seeds, lemongrass leaves, and langsat bark as botanical insecticide.

However, until today, there has been no report of scientific research on the effectiveness of the plant substance as a botanical insecticide. Based on the rationale outlined above, *Anopheles sp* resistance detection research will be carried out in Southeast Minahasa and the effectiveness and optimization laboratory analysis of insecticides power on mosquito in several plant substances used by the rural communities of Southeast Minahasa.

2. Materials and Methods

Tools and materials which is used in this research at each stage among others:

- Anopheles sp sample, plant botanicals which used as an insecticide on mosquito, obtained from the Tombatu District and Ratahan District, North Minahasa Regency.
- *Extraction*. The used chemicals are 90% ethanol, chloroform, deionized water and n-hexane. The used are oven, grinder, Philips blender, analytical balance, glass tools, Bunchi rotary.
- *Phytochemistry analysis.* The used chemicals are Dragendorff reagent, Mayer's reagent, Wagner reagent, 95% ethanol, HCl, Mg metals, Na_2CO_3, $FeCl_3$, H_2SO_4 and acetic anhydride, F254 silica gel TLC plate, 04 whatman filter paper. The used tools are test tubes, test tube racks, beakers, erlenmeyer, stirrer, hot plate and analytical balance.
- *Mosquito rearing.* The used materials and tools are bucket, water, and lamp.
- *Toxicity bioassay extracts:* plant extracts solution in various concentrations, glassware, and handsprayer.

2.1 Research Methods

This research uses laboratory experiment method using experimental design: Completely Randomized Design (CRD) multifactorial. The research consists of several stages.

2.2 Research Prosedure

1. **Mosquito Bionomic Study using WHO method**

   The observed parameter relates to blood-seeking behavior, resting behavior, breeding behavior. Mosquito mophology research is conducted in Laboratory of Parasitology Faculty of Medicine Sam Ratulangi University.

2. **Mosquitoes Catching in the Field** (Widiarti *et. al.* 2005)

   Mosquitoes catching are conducted in its native habitat (resting place), i.e. irrigation line and the swamp area which is protected from the sun at 5.00-8.00 o'clock and around cattle sheds at 22.00-24.00 o'clock.

   Mosquito identification is conducted based on Reid (1968), then are kept individually to be the first generation (F1)/iso female line. The early IV instar larvae of the first generation (F1) is used for treatment and biochemistry test.

3. **Mosquitoes Keeping in Laboratory**

   The catched mosquitoes lay their eggs in laboratory individually/iso female line. The procedures of this individual keeping are each mosquito is put separately each other to lay their eggs. After the eggs hatched into larvaes, each of them is moved to keeping tray which is 26 cm long and 15 cm wide. Everyday the larvae is given food which is floured meat mixed with rice bran in ratio 10:4 by 75 mg-200 mg, adjusted by the size of larvae instar (development stage). Once the larvae reach the fourth instar, the biochemical analysis is performed in laboratory.

4. **Extraction, phytochemistry analysis and insecticide test**

   The plant botanicals extraction use maceration method with various kinds of solvents such as ethanol, chloroform and n-hexane. The phytochemical analysis use the Harborne (1996) method which is modified with Thin Layer Chromatography (TLC) separation techniques. Insecticide test use toxicity method on mosquito larvae.

3. Result And Discussion

This research is conducted in some stages. Considering the climate and weather condition, then this research starts with a study of the malaria mosquito bionomic followed by a study of ethnomedical medicine plants which are potentially anti-mosquito (botanical insecticide mosquito). The limited equipment and the late arriving of chemical characterization make this study of mosquito resistance to insecticides that have been used to combat mosquitoes is conducted in the second year of this study.

3.1 Stage 1 Research: Bionomic Study of Malaria Mosquito Vector in Southeast Minahasa

The mosquito sample obtaining using catching method is conducted in two locations: Silian Village and Kali

Village, Toluaan Subdistrict, Southeast Minahasa. The selection of the villages is based on the data of malaria clinical cases in the villages that is high compared to other villages in Southeast Minahasa. Catching method is conducted usign outsider baits, inside baits, and around the cage. The catched mosquitoes show the mosquito species density variation based on the bait or catching location.

From the identification result, it is known that An. Vagus has the highest individual average number both in Lobu Village and Silian Village. In Lobu Village for UOL, the number of caught individuals of An. Vagus is 172, the UOD is 87 and the DD is 102, whereas the high population average number in Lobu Village but low in Silian Village. As well as An. Subpictus, An. Indifinitus is also found in a high number in Lobu Village, but low in Silian Village. Otherwise, An. Barbirostris is found in a high number in Silian Village but low in Lobu Village (Table 1).

Table 1 The Caught Species *Anopheles spp* Total in Lobu Village and Silian Village, Toluaan Subdistrict, Southeast Minahasa

Species	**Lobu Village**				**Silian Village**				**Total**
	UOL	**UOD**	**DD**	**KD**	**UOL**	**UOD**	**DD**	**KD**	
An. Barbirostris	15	7	8	34	653	542	175	345	1779
An. Tesselatus	12	1	5	28	32	10	7	320	415
An. Vagus	172	98	102	85	654	120	28	142	1401
An. Kochi	2	2	3	40	0	2	3	42	94
An. Aconitus	2	0	2	2	1	2	5	2	16
An. Indifinitus	83	45	43	342	4	2	1	8	528
An. Pediataeniatus	5	2	6	2	6	2	2	5	30
An. Subpictus	114	76	54	24	254	0	24	28	574
Total	**405**	**231**	**223**	**557**	**1604**	**680**	**245**	**892**	**4837**

Notes :

UOL : outsider bait

UOD : insider bait

DD : catching on wall

KD : catching around the cage

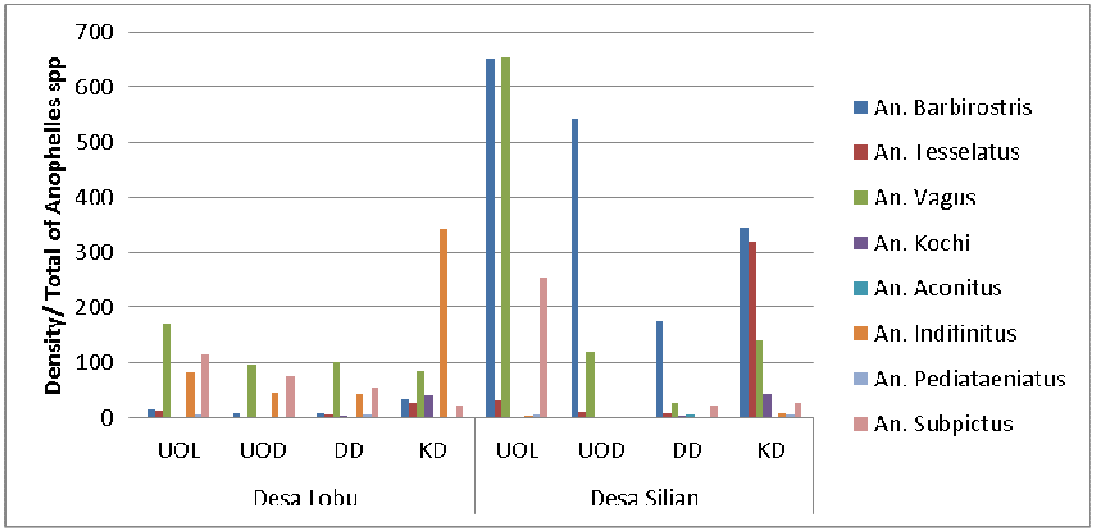

Figure. 1. Density of *Anopheles spp* in Lobu Village and Silian Village

An. Vagus and An. *Barbirostris* have the highest density of all species that are identified in UOL while for KD, An. *Barbirostris* and *An. Tesselatus* show the highest species density. From the species density graph, it is known

that species density is different based on the mosquito sampling subject, i.e. UOL, UOD, DD and KD. In Silian Village, An. *Barbirostris* and *An. Tesselatus* are more exophagic whereas *An. Vagus* is endophagic.

Table 2 shows the highest relative abundance in *An. Vagus.* In Lobu Village, 43.33% is caught in house wall (DD), 42.46% is caught using UOL, 42.42 % is caught using UOD and 15.26% is caught in KK. In Silian Village, the relative abundance of *An. Vagus* for UOL is 40.77%; UOD is 17.64%; DD is 11.42%; KD is 15.91% (Table 2).

Table 2 Relative Abundance of *Anopheles spp* Lobu Village and Silian Village, Toluaan Subdistrict, Southeast Minahasa

Species	Lobu Village				Silian Village				Total
	UOL	UOD	DD	KD	UOL	UOD	DD	KD	
An. Barbirostris	3.703704	3.030303	3.587444	6.104129	40.71072	79.70588	71.42857	38.67713	246.947887
An. Tesselatus	2.962963	0.4329	2.242152	5.02693	1.995012	1.470588	2.857143	35.87444	52.8621289
An. Vagus	42.46914	42.42424	45.73991	15.26032	40.77307	17.64706	11.42857	15.91928	231.661592
An. Kochi	0.493827	0.865801	1.345291	7.181329	0	0.294118	1.22449	4.70852	16.1133757
An. Aconitus	0.493827	0	0.896861	0.359066	0.062344	0.294118	2.040816	0.224215	4.37124793
An. Indifinitus	20.49383	19.48052	19.28251	61.40036	0.249377	0.294118	0.408163	0.896861	122.505735
An. Pediataeniatus	1.234568	0.865801	2.690583	0.359066	0.374065	0.294118	0.816327	0.560538	7.19506529
An. Subpictus	28.14815	32.90043	24.21525	4.308797	15.83541	0	9.795918	3.139013	118.342968
Total	100	100	100	100	100	100	100	100	**800**

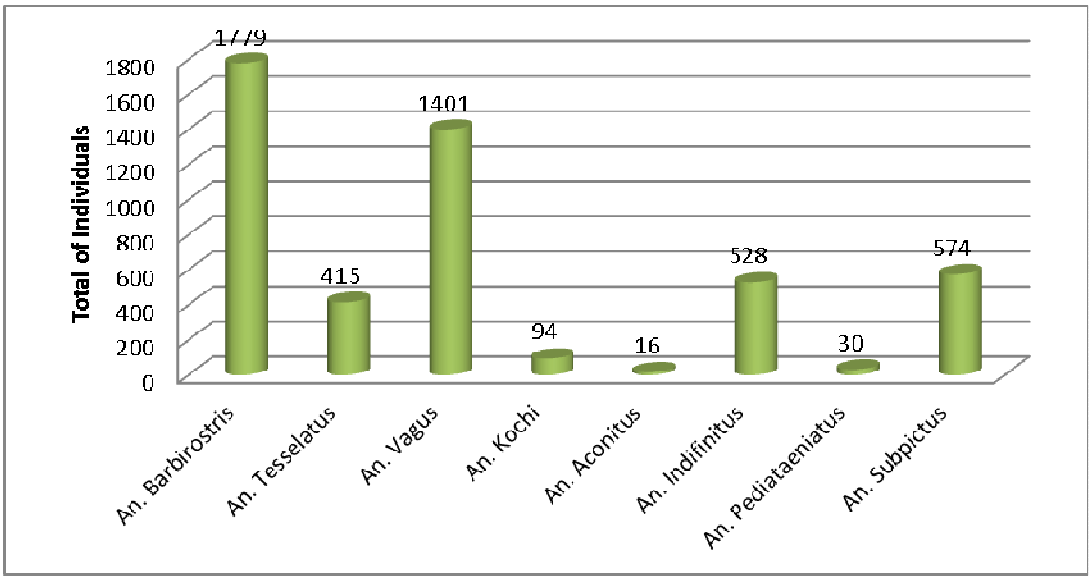

Figure 2. Total of Individu per Species in 2 sampling villages

The total of caught *An. Barbirostris* is 1779, An. Vagus is 1401, *An. Subpictus* is 574, An. Indifinitus is 528, *An. Tesselatus* is 415, *An. Kochi* is 94, *An. Pediataeniatus* is 30 and *An. Aconitus* is 16. Thus, there are 4 species with the highest relative abundance Lobu dan Silian Village, i.e. *An. Barbirostri, An. Vagus, An. Subpictus* and *An. Infinitus.*

The high of mosquito population in both villages is due to the two villages have many areas with standing water as mosquitoes nesting place. In Silian and Lobu Village, there are many ponds, rice growing areas where water is continuously available throughout the year despite the rainfall intensity is low.

In each area where there is malaria transmission, generally there are not more than three *Anopheles* species which become important factor. In Indonesia, 24 species which become malaria vectors has been found with their spreading that is described in the table above. *Anopheles* mosquito especially lives in tropical and sub-tropical areas but can live also in the medium climate area even in Arctic. *Anopheles* is rarely found in highland area which is more than 2000-2500 meters. Many *Anopheles* mosquitoes are found in lowland.

The female *Anopheles* mosquito bites on dusk with different frequence as their species. Consumption and rest habit of *Anopheles* mosquito can be classified as:

a. endophily: like to live in house; b. exophily: live to live outside; c. endophagy: bite in house; d. exophagy : bite outside; e. anthropophily : like biting human; f. zoophily : like biting animal. Flight distance of *Anopheles* mosquito is limited; it is usually not more than 2-3 km from its breeding place. If there is strong wind, *Anopheles* mosquito can be taken along until 30 Km. In additions, *Anopheles* mosquito can be taken along by plane or ships and spread malaria to non endemic areas.

Mosquito larva is found in almost area: settlement area, marsh and ponds that is in both villages. This causes the growth of mosquito population very high in these villages.

3.2 Stage 2 Research: Ethnobotanical And EthnomedicalAnti Mosquito Potential Plant Medicine Study In Southeast Minahasa

A. Extraction

Plant substance extraction is a very important stage in obtaining plant secondary metabolites which is used as medicine, cosmetics, and insecticides raw material. Extraction is conducted in maceration method that is to soak the plant simplicia at room temperature 1x24 hours for 3 days. The important factors that influence the extraction result are solvent, time, and temperature of the extraction.

There are many methods in exctrating plant substance, e.g. percolation methods, sokletation, and vapor destilation. Perlocation method is only good to be used in easily soluble organic compound, while sokletation is only good to be used in heat-resistant compounds. Therefore, maceration method is chosen so that the secondary compound isolation of the purple lemongrass and tembelek flower extract are maximal.

Table 3 The maceration of the purple lemongrass and tembelek flower extract and methanol

Extract	Macerated with methanol 1000 g extract			Methanol amount	Extract weight
	1st maceration	2nd maceration	3rd maceration		
Purple lemongrass	3000 ml	2500 ml	2000 ml	8000 ml	42.62 g
Tembelek flower	3000 ml	2500 ml	2000 ml	8000 ml	51.02 g

B. Phytochemistry Screening Analysis

Phytochemistry analysis is a method to know the secondary metabolite content in a plant example. In this research, phytochemistry analysis uses Harborne (1992). The analyzed compounds are alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids. The test result is shown in table 4.

Table 4 Qualitative Test Result (color) of Experiment Extract

Kind of Phytochemistry Test	**Purple Lemongrass Stem**	**Tembelek Flower Leave**	**Notes**
Alkaloids	+++	+++	+ = there are color
Flavonoids	+++	+++	
Tannins	+	++	- = no color
Triterpernoids	+++	+++	
Saponins	+	++	
Steroids	+++	++	

At table 4 it is shown that the value of almost sample is positive. It proves that generally experiment extract contains alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids. At the alkaloids test, the sample

with Mayer reagent substance in test tube produce white deposition, the sample testing with *Dragendrof* reagent substance shows red orange deposition and the sample testing with *wagner* reagent substance shows brown deposition. It shows that there is alkaloid in samples. This research result is appropriate with Harborne (1992) who says that if a solution is tested with *Mayer* reagent in test tube and produce white deposition, with *Dragendrof* reagent and shows red orange deposition, and with *wagner* reagent and shows brown deposition, so the sample contains alkaloids.

The flavonoid filtering test shows positive result that contains flavonoid. This is due to the sample testing produce red color after the magnesium (Mg) is added, and is followed with 10 drops HCL 5M adding in test tube which contains sample. This research result is appropriate with Harborn (1992), i.e. there is flavonoid in sample if it shows color change to red at the sample after is adeed with Mg and HCl.

Tannin filtering shows that from the galatine and ferric chloride addition it is found various results. At galatine addition, all samples show white, whereas at $FeCl_3$ addition only purple lemongrass that shows greenish color. It means the tannin in tested samples is in few consentration.

Saponins content test shows positive result. The saponin existence in material is seen through the foam forming in test tube. The more foam which is produced, the more the saponins in the material. Usually, the foam holds out for 10 minutes.

C. Active Ingredients Content Analysis

The analysis result with crude extract gas chromatography lantana camara is shown in the following table:

Table 5 KGSM Analysis Result for Tembelek (*Lantana camara*) Extract Sample

Peak	Retention Time	Abundance Percentage	Compounds Name
1	9,169	19,86	**2,3-Dihidro**
2	17,283	0,09	5-Bromo
3	17,740	72,07	**α-D-Galaktopiranosid**
4	19,916	1,13	Decanoic-acid
5	20,352	1,23	Hexadecanoic-acid
6	29,517	5,63	3,6-Bis(4-metoxiphenyl)

Based on Gas Chromatogprahy dan Mass Spectrophotometry analysis result for tembelek flower extract sample, there are six kinds of compounds that is in the extract where the two compounds which are the have highest abundance percentage are α-D-Galaktopiranosid and 2,3-Dihidro. α-D-Galaktopiranosid that amounts to 72.07 % is on the third retention peak. After that, 2,3-Dihidro with abundance percentage 19.86 % is on the first retention peak.

Based on Gas Chromatography and Mass Spectrophotometry analysis result for the purple lemongrass extract sample, there are 18 kinds of compound which is contained in the extract where the three compounds that have the highest abundance percentage are 11,14- eikosadienoic-acid, hexadecanoic-acid, dan Ergosta. 11,14-eikosadienoik-acid that amounts to 15,84% and 15.26% are at the ninth and eleventh retention peak. Then, hexadecanoic-acid with 11.17% abundance percentage is at the eighth peak. And Ergosta that amounts to 13.96 % is at ninth and sixteenth retention peak.

Table 6 KGSM analysis resut for Purple Lemongrass extract sample (*Cymbopogon citratus*)

Peak	Retention Time	Abundance Percentage	Compounds Name
1	13,269	3,14	Geranic-acid
2	16,618	2,90	Juniver kamvor
3	17,054	1,87	Viridifrol
4	18,325	2,66	Fenol
5	18,792	0,62	Etanona
6	19,024	4,16	Globulol
7	19,922	8,98	Hexadecanoic-acid
8	20,467	11,17	Hexadecanoic-acid
9	21,654	15,84	**11,14- eikosadienoic-acid**
10	21,910	1,77	Tetrakosanoic-acid
11	22,193	15,26	**11,14- eikosadienoik-acid**
12	26,933	2,12	9,12- octadekatrienoic-acid
13	31,217	2,32	13-metil
14	31,342	1,82	N-tert-butilmalimaid
15	31,533	1,45	26,27-dinorkolesta
16	32,106	13,96	**Ergosta**
17	33,496	6,05	Aristolenapoksida
18	34,162	3,92	Neptalena

D. Larvacyde Activity Analysis

Table 7 *Anopheles sp* Larvae Mortality at Purple Lemongrass Extract for 24 hours

Kind of Extract	Cosentration (ppm)	*Anopheles sp* Larva Mortality Number in 10 mosquitoes			Motality Total
		Repetition 1	Repetition 2	Repetition 3	
Chlorofom	1000	10	10	10	LC_{50} = 55.347
	500	9	10	9	
	100	7	9	8	
	10	5	5	4	
Asetil acetate	1000	10	10	10	LC_{50} =231.397
	500	7	6	6	
	100	3	3	2	
	10	0	0	0	
n-butanol	1000	10	10	10	LC_{50} = 540.116
	500	1	4	2	
	100	0	0	0	
	10	0	0	0	

The death of larvae is due to the larvae disability in phetoxiphicating the toxic compounds that enter into its body. Content toxicity of secondary metabolite at purple lemongrass extract gives toxicity effect on larvae that is seen through subletal symptoms to the larvae death. Based on the table above, the larvae mortality value of *anopheles sp* that is 50% of experiment animal is in 100 ppm dosis. It means that this dosis become the reference to determine the used dosis interval dosis on the real toxicity test.

Table 8 *Anopheles sp* Larvae Mortality at Temelek Flower Extract for 24 hours

Kinds of Extract	**Concentration (ppm)**	***Anopheles sp* Larva Mortality Number in 10 mosquitoes**			**Mortality Total**
		Repetition 1	**Repetition 2**	**Repetition 3**	
Chloroform	1000	10	10	10	LC_{50} = 43.0730
	500	9	9	9	
	100	8	9	7	
	10	5	4	4	
Asetil acetate	1000	10	9	10	
	500	9	7	7	LC_{50} = 120.420
	100	6	4	2	
	10	4	2	1	
n-butanol	1000	9	10	8	LC_{50} = 872.160
	500	2	1	2	
	100	0	0	0	
	10	0	0	0	

The death of larvae is due to the larvae disability in phetoxiphicating the toxic compounds that enter into its body. Content toxicity of secondary metabolite at tembelek flower extract gives toxicity effect on larvae that is seen through subletal symptoms to the larvae death. Based on the table above, the larvae mortality value of *anopheles sp* that is 50% of experiment animal is in 90 ppm dosis. It means that this dosis become the reference to determine the used dosis interval dosis on the real toxicity test.

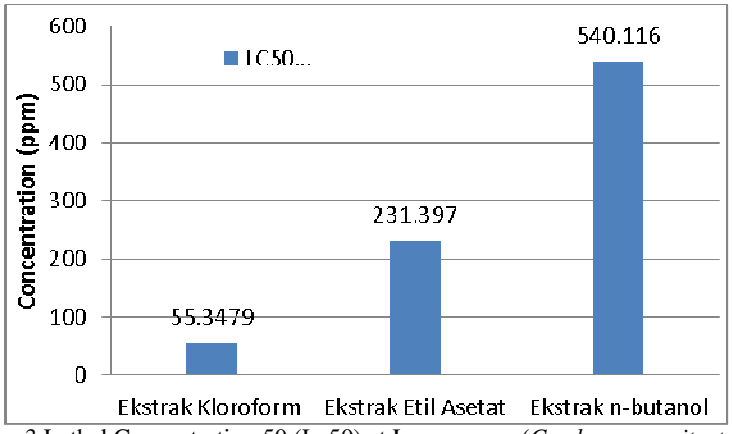

Figure 3 Lethal Concentration 50 (Lc50) at Lemongrass (*Cymbopogon citratus)* Extract

The test result above shows the difference of death *Anopheles sp* larvae number at various concentrations. It shows there is increasement of death *Anopheles sp* number along with the incereasement of purple lemongrass concentration. The purple lemongrass chloroform extract shows the very good anti-mosquito activity with the very low LC_{50} value which is 55.3479 ppm. The lower the concentration, the better it is. Chloroform ($CHCl_3$) which is a non polar solvent with the boiling point of 61^0 C pull non polar compounds in purple lemongrass essential oils. While in ethyl acetate which is a semi-polar solvent with a boiling point of 77^0C, the LC_{50} value

amounts to 231.397 ppm. N-butanol is a polar compound with a boiling point of 118^0C, the LC_{50} value amounts to 872.16. So it can be seen that the best concentration in purple lemongrass is chloroform ($CHCl_3$) extract that is 55.3479 ppm.

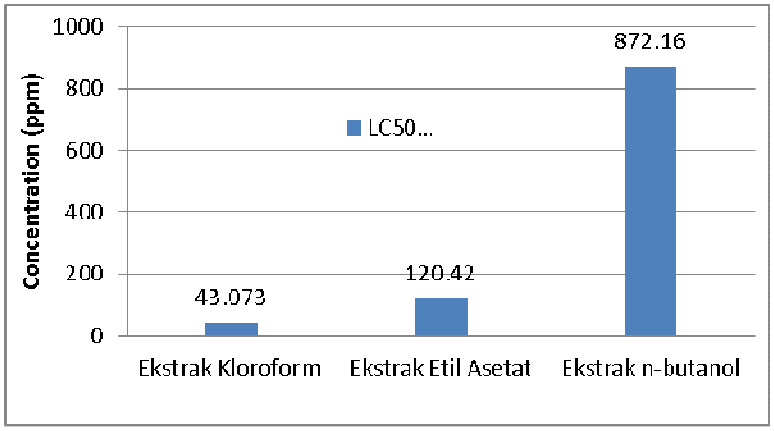

Figure 4 Lethal Concentration 50 (Lc50) at Tembelek (*Lantana Camara*) Flower Extract

The test result above shows that there is difference in the death *Anopheles sp* larvae number at various contentration. It is shown the death *Anopheles sp* larvae number along with increasement of tembelek flower concentration. The tembelek flower chloroform extract shows the very good anti-mosquito activity with very low LC_{50} value that is 43,073. The lower the concentration, the better it is. Chloroform ($CHCl_3$) is non polar solvent with a boiling point of 61^0 C that pulls non polar compounds in tembelek flower essential oil. In the ethyl acetate which is a semi-polar solvent with a boiling point of 77^0C, the LC_{50} value amounts to 120.42. N-butanol is a polar compound with a boiling point of 118^0C, the LC50 value amounts to 872.16. So it can be seen that the best concentration in tembelek flower is chloroform ($CHCl_3$) extract that is 43.073 ppm.

E. Extract Toxicity Contents Analysis on Mosquito

Extract Toxicity Contents Analysis on Mosquito *in vivo* is analyzed based on mosquito mortality after treated extract per unit time (Table 9).

Table 9 *Anopheles sp* that amounts to 50 mosquitoes at purple lemongrass

Kind of Extract	Concentra-tion ppm	Time	Mortality number of *Anopheles sp*		
			Repetition 1	Repetition 2	Repetition 3
Chlorofom	100	10 minutes	28	28	29
		5 minutes	34	32	33
	10	10 minutes	11	13	12
		5 minutes	18	18	19
ethyl acetate	100	10 minutes	22	24	22
		5 minutes	27	25	28
	10	10 minutes	5	7	5
		5 minutes	15	12	12
N-butanol	100	10 minutes	0	0	0
		5 minutes	0	0	0
	10	10 minutes	0	0	0
		5 minutes	0	0	0

Table 10 *Anopheles sp* Mosquito Toxicology Content Analysis with 50 mosquitoes at Tembelek Flower

Kind of Extract	Concentra-tion ppm	Time	*Anopheles sp* mosquito mortality number		
			Repetition 1	Repetition 2	Repetition 3
Chlorofom	100	10 minutes	29	30	29
		5 minutes	37	37	39
	10	10 minutes	9	7	7
		5 minutes	15	12	15
ethyl acetate	100	10 minutes	19	19	18
		5 minutes	23	22	22
	10	10 minutes	4	2	5
		5 minutes	11	9	11
N-butanol	100	10 minutes	0	0	0
		5 minutes	0	0	0
	10	10 minutes	0	0	0
		5 minutes	0	0	0

3.3 Discussion

From the research result, it is known that the bigger the concentration of the used purple lemongrass *(cymbopogon citratus)* and *tembelek flower (lantana camara)* are, the more the death larvae and it is found the LC_{50} on *Anopheles sp* larvae is on % concentration. The number variation of death larvae at each repetition with same concentration is caused by the sensitivity of each different experiment larvae that probably relates to the existence of larvae's resistant power on certain toxic exposure.

Based on the result of *One-way Anova* analysis, *Post Hoc* test, c*orelation and regression test*, the cause of *Anopheles sp* larvae death is allegedly the active substances contained in purple lemongrass *(cymbopogon citratus)* and *tembelek* flower *(lantana camara)*. Purple lemongrass *(cymbopogon citratus)* and *tembelek* flower *(lantana camara)* contain essential oil compounds (α-D-Galaktopiranosid, 2,3-dihydro, 11:14 eikosadienoik-acid, acid hexadecanoic, and Ergosta), alkaloids, flafonoid, tannins, terpenoids, saponins, and steroids that are effective as larvicides. Essential oils are volatile compounds that do not dissolve in water that comes from plants. Essential oils can be separated from the plant tissue through the distillation process.

The observation on toxicity test are the larvaes often rise to the surface and the frequency is very long which indicates that the need for dissolved oxygen in water decreases so that the larvaes frequently go to the surface to meet the needs of oxygen; the other symptom is reduced response to stimuli characterized by the decrease of aggressiveness when it is touched. This situation as the active symptoms is caused by bioactive compounds in extracts of purple lemongrass stems and tembelek flower leaves which are toxic, such as alkaloids, flafonoid, tannins, terpenoids, saponins, and steroids. Toxic compounds which are contained within these extracts are foreign substances for the larvae body. These substances can enter the body through several larval body parts, such as the surface of the body wall, airways, and the digestive tract of food.

Wall surface of the body is the outer part of larval body that can absorb large amounts of insecticide because this section is directly related to pesticides. In the larval breathing, air and oxygen enter to the trachea by diffusion with the aid of abdominal movement. In addition, the toxic substances in the extract of the purple lemongrass stalk and tembelek flowers leaves can also enter the respiratory system in the form of gas or fine grain which is brought into living tissue.

In this research, the toxic substance enter into the mouth of larvae through the respiration system which is spiracle in the body surface and cause the nerves weak, and damages on spiracle, consequently the larvae cannot breathe and die. The cause of the weak nerves is saponin compounds. It is due to the saponin compounds can inhibit the action of the enzyme acetylcholinesterase. Aseti lkolin which is formed by the central nervous system function is to conduct impulses from nerve cells to muscle cells. Once the impulse is delivered, the process is stopped by the acetylcholinesterase enzyme that breaks down acetylcholine into acetyl co-A and choline. The

existence of insecticide compounds (alkaloids, and saponins flafonoid) will inhibit the operation of this enzyme resulting in accumulation of acetylcholine which would lead to chaos in the delivery system impulses to the muscles which can result in muscle spasms, paralysis and ends to death.

Flavonoid compounds can be described as a sequence of C6 - C3 - C6 . that is, the carbon skeleton consisting of two groups C6 (substituted benzene ring) connected by a three - carbon aliphatic chain . Different classes in this group are distinguished by additional heterocyclic ring - oxygen and hydroxyl groups that spread according to different patterns . Flavonoids are found as glycosides . Largest class of flavonoids characterized annex connecting the ring has a three - carbon chain with one of the benzene ring (Robinson , 1999) . Effects of flavonoids against a variety of organisms . One of them is also a potent inhibitor of respiration . Components that interfere with energy metabolism have been identified either from natural or synthetic sources . Disorders of energy metabolism occurs in the mitochondria by inhibiting electron transport system or by blocking the transport system with the coupling between ATP production . Barriers to block the electron transport system ATP production and cause a decrease in oxygen consumption by the mitochondria . Respiratory inhibitors work by inhibiting the respiratory chain , inhibiting oxidative phosphorylation or by disconnecting the circuit (uncouple) between the respiratory chain phosphorylation oksidati . Inhibiting electron transport at site I work by inhibiting coenzymes Q reductase (NADH oxidase inhibitor) . At site II inhibitors by inhibiting cytochrome bc complex.

C*oupling* barriers causes transport system running normally, but the production of ATP from the electron transport process can not be combined. This is due to the damage of mitochondrial integrity so that the proton gradient for passing through the inner mitochondrial membrane is lost. With the coupling resistance, oxygen consumption increased, but ATP is not produced. The energy metabolism disorders and ATP loss cause slow toxicity and effect on all components including paralysis and death of the larvae. Kaempherol, myricetin, and quercetin are included to the flavonoids group, i.e. flavonols. Flavonoids are good reducing agents that can inhibit the oxidation reaction, both enzymes and non-enzyme (Robinson, 1995). Enzimatically, *Flavonoid* inhibits the oxidation process by working as an ATP-ase inhibitors, NADH-oxidase-inhibitor (coenzymes Q reductase inhibitors) and cytochrome inhibitors. The existence of NADH-oxidase-inhibitor (coenzymes Q reductase inhibitors) leads to the electrons flow block from NADH to CoQ (coenzymes Q). Cytochrome inhibitors block the flow of electrons from cytochrome b to cytochrome c1. By inhibition of electron flow from cytochrome b to cytochrome c1, it leads all electron acceptors before cytochrome b is reduced. *ATP-ase inhibitor* works on *site V,* that is by inhibiting the ATP synthetic catalyst from ADP.

Mitochondrial membrane damage which is caused by flavonoids may occur because of Phospolipase inhibitor. Phospolipase inhibitors results in impaired mitochondrial membrane integrity and signal transduction so that the proton gradient to pass through the inner membrane of mitochondria is lost. Mitochondrial damage in non-enzymatic is suspected because of Flavonoids cytotoxic properties.

Larvicides mechanism of tannins associated with its ability to inactivate adenosine, enzymes and cell proteins. Hydrolyzable tannin is usually in the form of amorphous compounds, hygroscopic, brown yellow and soluble in water (especially hot water) to form a colloidal solution that is not the actual solution. The more pure the tannin, the less solubility in water and more easily obtained in crystalline form it is. Reaction of tannins with proteins is unique and depends on the structure of tannins. Some tannins have been proven having activity in inhibiting reverse transcriptase enzyme and DNA topoisomerase (Robinson, 1999). Tannins bind to polysaccharides and water soluble. Catechin tannins play an important role as larvicides for causing damage to the cell membrane so that mosquito larvae die. The role of flavonoids larvicida occurs through a resistance mechanism of larvae nucleic acid synthesis (DNA), which causes the death of the larvae.

4. Conclusion

From this research result, it can be concluded that:

1. There are 8 species of Anopheles spp which are found in Lobu and Silian Village, Toluaan Sub-district, Southeast Minahasa.
2. The total of catched mosquitoes: An. Barbirostris is 1779, An. Vagus is 1401, An. Subpictus is 574, An. Indifinitus is 528, An. Tesselatus is 415, An. Kochi is 94, An. Pediataeniatus is 30 and An. Aconitus is 16.
3. There are 4 species with the highest abundance in Lobu and Silian Village, i.e. An. Barbirostri, An. Vagus, An. Subpictus and An. Infinitus.
4. Treatment activity of purple lemongrass oil (Cymbopogon citratus) and Tembelek (Lantana camara) are

effective as an ethnobotany anti mosquito plant extracts in Southeast Minahasa, North Sulawesi.

5. There is plant extract phytochemistry class content and plant extract combination. And the toxicity level (*Knockdown time* [KT] and *lethal time* [LT_{90}]) of anti mosquito plant insecticyde.
6. The active ingredient which is particularly contained in purple lemongrass essential oil (**α**-D-Galaktopiranosid) affects much in controlling the activity of anti-mosquito *anopheles sp.*

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