Mosquito Larvicides of Partial and Combinations Extract of Ethnobotanical Plant from North Sulawesi, Indonesia

by Yermia Mokosuli

http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.911.921

Research Article

Mosquito Larvicides of Partial and Combinations Extract of Ethnobotanical Plant from North Sulawesi, Indonesia

[1]Carolin Manuahe, [2]Mokosuli Yermia Semuel, [1]Ellen Hettie Adil and [2]Orbanus Naharia

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Manado State University, FMIPA UNIMA Campus, Tonsaru Tondano, North Sulawesi, Indonesia
[2]Laboratory of Bioactivity and Molecular Biology, Faculty of Mathematics and Natural Sciences, Manado State University, Tonsaru Tondano, North Sulawesi, Indonesia

Abstract

Background and Objective: Diseases caused by microbes vectored by mosquitoes are still a health problem in tropical countries today. DHF and Malaria are the two primary diseases vectored by mosquitoes, the morbidity and mortality rates have increased in low countries until now. However, the best way to control these two diseases is to control vectors, namely mosquitoes. Research has been conducted to determine the bioactive content and larvicidal activity of local plant extracts of North Sulawesi. **Materials and Methods:** The clove and trumpet flower samples were obtained from Minahasa, while the nutmeg samples were obtained from Sitaro Regency. Empirically, people use plant parts to repel mosquitoes. Extraction of plant simplicia was carried out by the maceration method. Qualitative and quantitative methods carried out the phytochemical content analysis. Qualitative analysis uses Harborne's (1996) method while qualitative analysis uses the UV Vis Spectrophotometer method. Toxicity tests were carried out on mosquito larvae developed in the laboratory. **Results:** The results showed that combining clove leaf extract, nutmeg flesh extract and trumpet flower synergistically increased the bioactive content. Flavonoids increased in the combination of extracts compared to partial extracts. The combination of extracts showed the highest toxicity to mosquito larvae (LC_{50}: 22.541 mg L^{-1}), while the lowest was the partial extract of clove leaves with LC_{50} (54.965 mg L^{-1}). **Conclusion:** The combination of extracts showed the best toxicity activity on mosquito larvae. Research on bioactive characteristics and toxicity in adult mosquitoes needs to be carried out in the future.

Key words: Clove leaf, nutmeg flesh, trumpet flower, extract, larvicides, toxicity, mortality

Citation: Manuahe, C., M.Y. Semuel, E.H. Adil and O. Naharia, 2022. Mosquito larvicides of partial and combinations extract of ethnobotanical plant from North Sulawesi, Indonesia. Pak. J. Biol. Sci., 25: 911-921.

Corresponding Author: Mokosuli Yermia Samuel, Laboratory of Bioactivity and Molecular Biology, Faculty of Mathematics and Natural Sciences, Manado State University, Tonsaru Tondano, North Sulawesi, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mosquito population control is still a global public health priority. Mosquitoes are vectors of various infective diseases with high fatality rates such as Malaria, febrile fever, chikungunya, yellow fever, Zika and West Nile, affecting a significant proportion of the world population[1-3]. These diseases contribute significantly to an estimated 17% of the global vector-borne disease burden of all infectious diseases, accounting for >1 billion new cases and >1 million deaths annually[4]. Mosquito-borne diseases contribute significantly to the worldwide burden of disease, mortality, poverty and social disadvantage[5,6].

Malaria and DHF are endemic diseases in Indonesia, which until now have increased morbidity and mortality[7]. In 2020, in the COVID-19 pandemic situation, there was an increase in dengue cases in North Sulawesi, Indonesia, compared to the previous year. Climate change has affected the adaptation of mosquitoes to the environment[8-11]. Besides, the use of synthetic insecticides against mosquitoes also raises resistance and resurgence[12,13]. Based on research reports and empirically, several liquid synthetic insecticides have decreased toxicity to adult mosquitoes. Besides, residues of synthetic insecticides can be toxic and carcinogenic to humans[14].

The use of plants as bioactive insecticides in the tropics is based on biological interactions between plants and insects[15-17]. Plants produce certain compounds as a form of self-defence from microbial attack or multicellular organisms, especially insects[18]. Some plants reported containing bioactive insecticide compounds include *Acer rubrum* L. (Aceraceae), *Betula alleghaniensis* Britton (Betulaceae), *Betula papyrifera* Britton, *Carya cordiformis* K. *Prunus serotina* Ehrh (Rosaceae), etc.[19], langsat seeds[20]. Furthermore, *Nicotiana tabacum*, *Ocimum basilicum*, *Datura stramonium*, *Chenopodium album* and *Cassia fistula* have strong larvicidal activity against *Culex quinquefasciatus*. Acetate extract of *Acacia auriculiformis* fruit showed high mortality against mosquito larvae at a concentration of 300 ppm[21]. *Ricinus communis* seed extract has better larvicidal activity than a leaf. Both extracts can be used as an effective larvicide against mosquitoes[22].

As a malaria-endemic area, the Minahasa people of North Sulawesi, Indonesia, have long recognized and used certain plant species as botanical insecticides. Clove oil is used as a mosquito repellent by the Minahasa tribal community. Nutmeg leaves are also used as a mosquito repellent by the Sanger tribe. In contrast, the trumpet flower has a distinctive aroma that is not liked by mosquitoes. However, research on the toxicity of partial extracts and combinations of these three plant species, specifically from the Wallacea Zone, is still very little reported.

Controlling mosquito populations at the larval stage is more likely than adult mosquitoes[23]. The use of partial plant extracts has been widely carried out. However, the activity of the combination of plant extracts as larvicides is still reported. The combination of plant extracts in this study was carried out on several ethnomedical plants used by the local community of North Sulawesi as a source of bioactive mosquito repellent for generations. These plants are clove leaves, nutmeg flesh and trumpet flowers.

Research has been carried out to obtain effective concentrations of partial plant extracts and combinations of plant extracts against mosquito larvae.

MATERIALS AND METHODS

Study area: The research was conducted at the Bioactivity and Biopharmaceutical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Manado State University, Indonesia. The research was conducted from March to September, 2021.

Plant samples: Nutmeg (*Myristica fragrans* L.) was obtained from Siau, Sitaro District, North Sulawesi. Clove leaves (*Syzygium aromaticum* L.) were obtained from Kombi District, Minahasa Regency, North Sulawesi. White trumpet flower (*Brugmansia suaveolens* L.) was obtained from West Tondano District, Minahasa Regency, North Sulawesi. The samples obtained were labelled and preserved in the sample box.

Tools and materials: The tools used in this research include the Blender Philips (Belanda), Kern analytical balance, Rotary Evaporator Buchi (Switzerland) to evaporate the solvent and get the crude extract, Eppendorf centrifuge (Germany), Eppendorf micropipette (Germany), PerkinElmer UV Vis spectrophotometer (USA), Hirox KH8700 microscope (Japan), Mummert hot plate (Germany), glassware Pyrex and others. While the ingredients used are clove leave extract, nutmeg flesh extract, trumpet flower extract, ethanol pa Merck, chloroform pa Merck, ethyl acetate pa Merck, Butanol pa Merck, ion-free water, standard quercetin Brand, Dragendorff reagent, Mayer's reagent, Wagner's reagent, HCl, Mg metal, Na_2CO_3, $FeCl_3$, H_2SO_4 and acetic anhydride, etc.

Research procedure: The research was carried out in four stages: The preparation of mosquito test objects, plant extraction, analysis of bioactive content, formulation of test extracts and larvicide testing.

Preparation of mosquitoes as larvicide test objects

Catching mosquitoes in the field (Sayono *et al*.[23]): Mosquito catching is carried out in their natural habitat (resting place), namely irrigation canals and swamp areas protected from sunlight, in the morning from 05.00-08.00 and around cattle pens from 22.00-24.00. Identification of mosquitoes was carried out by piercing the Book Reid (1968) and then reared individually into the first generation (F1)/iso female line. The first generation (F1) of early IV instar larvae was used for treatment and biochemical tests.

Mosquito maintenance in the laboratory: The captured mosquitoes are then spawned in the laboratory individually/iso female line. This individual maintenance is that each mosquito is placed separately from one another to lay eggs. After the eggs hatched into larvae, each was transferred to a rearing area, namely, a tray measuring 26 and 15 cm long. Every day the larvae are given food in the form of fish pellets. The subjects that will be used are mosquito larvae of Anopheles sp stage 3 and 4. After becoming mosquitoes, they are fed with a mixture of bran powder and meat in a ratio of 10:4 as much as 75-200 mg.

Extraction and analysis of bioactive content

Plant extraction: Extraction has been done by the maceration method by Kaunang and Semuel[24] and Semuel *et al*.[25]. The ratio of solvent and solute is 1:4 (250 g of plant extract is macerated in 1000 mL of solvent). Extraction was carried out at room temperature for 3×24 hrs. The solvent used is 70% ethanol.

Bioactive content analysis

Phytochemical analysis (Kaunang and Semuel)[24]

Alkaloid test: A total of 0.1 g of the extract was added to 3 mL of chloroform and three drops of ammonia. The chloroform fraction was separated and acidified with ten drops of 2 M H_2SO_4. The acid fraction was taken and then Meyer and Wagner's reagent was added. The presence of alkaloids was indicated by the formation of a white precipitate by the Meyer reaction and a brown precipitate by the Wagner reaction. As a comparison, use the blood footprint.

Saponin and flavonoid test: A total of 1 g of extract was put in a beaker, then added to 100 mL of hot water and boiled for 5 min, after that, it was filtered and the filtrate was used for testing. The saponin test was carried out by shaking 10 mL of the filtrate in a closed test tube for 10 sec and then leaving for 10 min. The formation of stable foam indicates the presence of saponins. Another 10 mL of the filtrate was added with 0.5 g of magnesium powder, 2 mL of carbohydrate alcohol (a mixture of 37% HCl and 95% ethanol in a ratio of 1:1) and 20 mL of amyl alcohol then shaken vigorously. The formation of red, yellow and orange colours on the amyl alcohol layer indicates the presence of flavonoids.

Tannin test: A total of 0.1 g of the extract was added to 2 mL of water and then boiled for several minutes. Then filtered and the filtrate was added with one drop of 1% $FeCl_3$ (w/v). Dark blue or greenish-black colour indicates the presence of tannins.

Triterpenoid and steroid test: A total of 0.1 g of the extract was added to 2 mL of 30% ethanol, then heated and filtered. The filtrate was evaporated and then 1:1 ether was added. The ether layer was added with Lieberman Burchard's reaction (3 drops of acetic anhydride and one drop of concentrated H_2SO_4). Red and green colours indicate the presence of triterpenoids and green colours indicate the presence of steroids.

Quantitative analysis of flavonoid content

Determination of the maximum wavelength (max) of Quercetin: The maximum wavelength of Quercetin by running the quercetin solution at a wavelength of 400-450 nm. The result of running is the maximum wavelength of the quercetin standard, which is 435 nm. The wavelength was used to measure the absorbance of the combination of clove leaf extract:nutmeg pulp:trumpet flower (1:1:1).

Quercetin standard curve: A total of 25 mg of standard Quercetin (Merck) was dissolved using 25 mL of absolute ethanol. The stock solution was pipetted as much as 1 mL and the volume was made up to 10 mL with ethanol to obtain a concentration of 100 ppm. From a standard solution of 100 ppm quercetin, then several concentrations were made, namely 6, 8, 10, 12 and 14 ppm. From each concentration of the standard solution of Quercetin, 1 mL was pipetted. Then 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. The absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 435 nm[26].

Determination of the total flavonoid content of the extract: Weighed 15 mg of extract, dissolved in 10 mL of ethanol, to obtain a concentration of 1500 ppm. About 1 mL of this solution was pipetted and then 1 mL of 2% $AlCl_3$ solution and 1 mL of 120 mM potassium acetate were added. Samples were incubated for 1 hr at room temperature. The absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 435 nm. Samples were made in three replications for each analysis and the average value of absorbance was obtained[26].

Larvicide test

Exploration test: An exploratory or preliminary test was conducted to determine the treatment concentration that caused the death of 50% of the total population of larvae tested. The criteria for death are test animals or larvae that do not move and, if touched, do not react.

Larvicide activity analysis: *Anopheles* sp., larvae, as the target test animals, have integuments that are easily damaged[24]. Therefore, the method used is the dipping method. The test animals are immersed or put into the test solution. The test is carried out at room temperature.

Data analysis: The data from the extraction and analysis of phytochemical groups were analyzed descriptively. The flavonoid content was determined by regression analysis. Lethal Concentration 50 (LC_{50}) was determined based on probit analysis of data from the larvicide test results.

RESULTS

Plant extraction: Fresh samples of clove leaves were obtained from the Minahasa Clove Kombi plantation, Minahasa Regency, North Sulawesi, Indonesia. Nutmeg samples were obtained from Siau Sitaro Regency, North Sulawesi, Indonesia. While the trumpet flower samples were obtained from Kasuang, Minahasa Regency, North Sulawesi, Indonesia. The weight of fresh samples that have been mashed is 250 for each type of plant Simplicia is macerated with 70% ethanol for 2×24 hrs. Evaporation of the solvent with a rotary evaporator produces a blackish-brown extract. Each type of extract has a distinctive aroma, such as cloves, nutmeg and flowers (Fig. 1a-c). The highest average weight of the extract was shown by the nutmeg flesh extract, which was 11.57 with a yield percentage of 4.63. While the lowest average weight of the extract was indicated by the extract of the trumpet flower, which was 4.85 g with a percent yield of 1.94 (Table 1).

Phytochemical content analysis: Based on the intensity of the colour and precipitate obtained from the analysis of the content of the phytochemical groups, it was obtained that the combination of extracts showed the content of the groups with better intensity than the partial extracts. However, the three types of extracts showed flavonoid content with the same intensity as the combination of extracts. Trumpet flower and nutmeg flesh showed relatively the same intensity of alkaloid and triterpenoid content (Table 2).

Table 1: Extract weight and percent yield of different extracts

Extract	Replication	Samples weight (g)	Solvent volume (mL)	Extract weight (g)	Yield (%)	Average extract weight (g)	Average yield (%)
Clove leaves	1	250	1000	10.09	4.04	9.87	3.95
	2	250	1000	10.21	4.08		
	3	250	1000	9.32	3.73		
Nutmeg flesh	1	250	1000	11.16	4.46	11.57	4.63
	2	250	1000	12.32	4.93		
	3	250	1000	11.24	4.50		
Trumpet flower	1	250	1000	4.56	1.82	4.85	1.94
	2	250	1000	4.45	1.78		
	3	250	1000	5.53	2.21		

Table 2: Qualitative test results (colour and precipitate) extract

Types of phytochemical test	Clove leaves	Trumpet flower	Nutmeg flesh	Extract combination (1:1)	Test indicators
Alkaloid	+	++	+	++	Meyer's reaction: White precipitate Dragendorff's reaction: Orange precipitate Wagner's reaction: Brown precipitate
Flavonoid	++	++	++	++	Reddish colour after the addition of magnesium powder and followed by the addition of 10 drops of 5 M. HCl
Tanin	+	+	+	++	Addition of gelatin and $FeCl_3$, brownish green
Saponin	+	+	+	++	Stable foam for 5-10 min
Triterpenoid	+	++	++	++	Red or purple colour indicates the presence of triterpenoids)
Steroid	+	+	++	++	Green or blue colour indicates the presence of steroids)

+: Contains a group of phytochemical compounds indicated by colour indicators and/or precipitates formed and ++: Contains a group of phytochemical compounds in high intensity indicated by colour indicators and/or precipitates formed

Total flavonoid content: In this study, the standard flavonoid Quercetin (Merck) was used. Quercetin belongs to the flavonoid group of flavonols with a keto group at atomic C number 4 and an OH group at atomic C number 3 and atomic C number 5. Maximum absorption is determined at a wavelength distribution of 400-455 nm. The results of running the optimum absorption of quercetin standard at a wavelength of 435 nm. This wavelength was used to measure the uptake of the extract samples (Table 3).

The standard yield of Quercetin obtained was plotted between the concentration and absorbance. A linear regression equation was obtained, namely, y = 0.049x-0.006 with an R^2 value of 0.9976 and an R-value of 0.997. The quercetin calibration curve equation can be used as a comparison to determine the concentration of total flavonoid compounds in the sample extract (Fig. 2). As a control, a blank aqua dest solution was used. Based on the determination of the total flavonoid content of each extract, the highest flavonoid content was obtained in the combination of extracts with an average of 10.835 mg L^{-1}. The partial extract that showed the highest average flavonoid content was cleft leaf extract, 6.158 mg L^{-1}. Meanwhile, the extract with the lowest moderate flavonoid content was trumpet flower extract, 4.268 mg L^{-1} (Table 4).

Larvicide test: After 24 hrs of giving the extract to the test larvae, it showed the highest mortality rate of the three extracts at the test concentration of 1000 ppm. The trumpet flower extract indicated the lowest mean mortality at a concentration of 10 ppm (Table 5).

The combined extract mortality test was performed three times. Each concentration was repeated three times. The highest mortality rate was at a concentration of 1000 ppm, while the lowest was ten ppm (Table 6).

The determination of the lethal concentration 50, i.e., the total population of 50% dead larvae, was carried out by probit analysis. The highest LC_{50} was shown in the extract combination (1:1:1), 22.541 mg L^{-1}, while the lowest was in the clove extract, 54.965 mg L^{-1} (Fig. 3).

Table 3: Results of the absorbance measurement of the standard solution of Quercetin at a maximum wavelength of 435 nm

Concentration	Absorbance (nm)
6	0.294
8	0.386
10	0.475
12	0.576
14	0.689

Table 4: Results of the determination of the total flavonoid content % (w/w) in the extract using the standard Quercetin

No	Extract	Replication	Total flavonoid level (mg L^{-1})	Average
1	Trumpet flower	1	3.657	4.268
		2	3.453	
		3	5.695	
2	Nutmeg flesh	1	6.967	5.508
		2	5.706	
		3	3.851	
3	Clove leaf	1	4.842	6.158
		2	7.720	
		3	5.912	
4	Combination of extracts (1:1:1)	1	11.069	10.835
		2	10.616	
		3	10.822	

Table 5: Mosquito larvae mortality ethanol extract

			Number of dead mosquito larvae				
Extract	Concentration (ppm)	Number of larva	1	2	3	Total dead	STDEV
Clove leaf	1000	10	10	9	9	28	0.577
	500	10	7	9	8	24	1.000
	100	10	7	7	8	22	0.577
	10	10	3	2	3	8	0.577
Nutmeg flesh	1000	10	10	10	9	29	0.577
	500	10	8	8	9	25	0.577
	100	10	8	7	6	21	1.000
	10	10	2	2	3	7	0.577
Trumpet flower	1000	10	10	10	10	30	0.000
	500	10	9	9	9	27	0.000
	100	10	8	8	8	24	0.000
	10	10	3	3	3	9	0.000

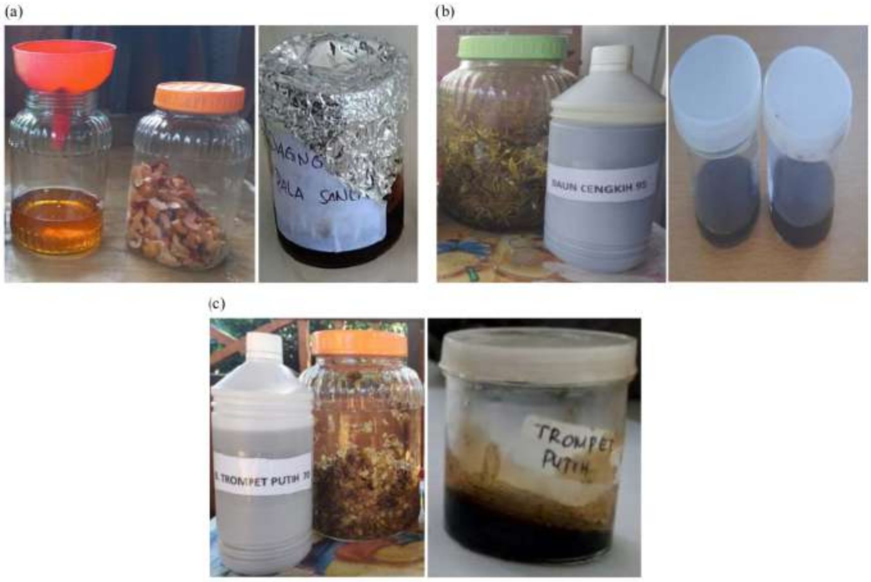

Fig. 1(a-c): Extraction of (a) Nutmeg flesh, (b) Clove leaves and (c) Trumpet flower ethanol extract

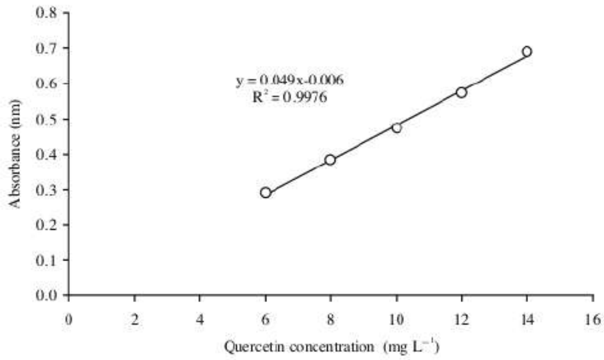

Fig. 2: Quercetin standard curve for total flavonoid compounds in the sample extract

Table 6: Mortality of mosquito larvae in extract combinations

Extract replication	Concentration (ppm)	Number of Larva	Number of dead mosquito larvae			Number of death	STDEV
			1	2	3		
U_1	1000	10	10	10	10	30	0.000
	500	10	9	10	9	28	0.577
	100	10	9	9	9	27	0.000
	10	10	5	5	3	13	1.155
U_2	1000	10	10	10	10	30	0.000
	500	10	8	9	9	26	0.577
	100	10	8	8	8	24	0.000
	10	10	1	0	1	2	0.577
U_3	1000	10	10	10	9	29	0.577
	500	10	9	8	9	26	0.577
	100	10	9	9	8	26	0.577
	10	10	5	5	1	11	2.309

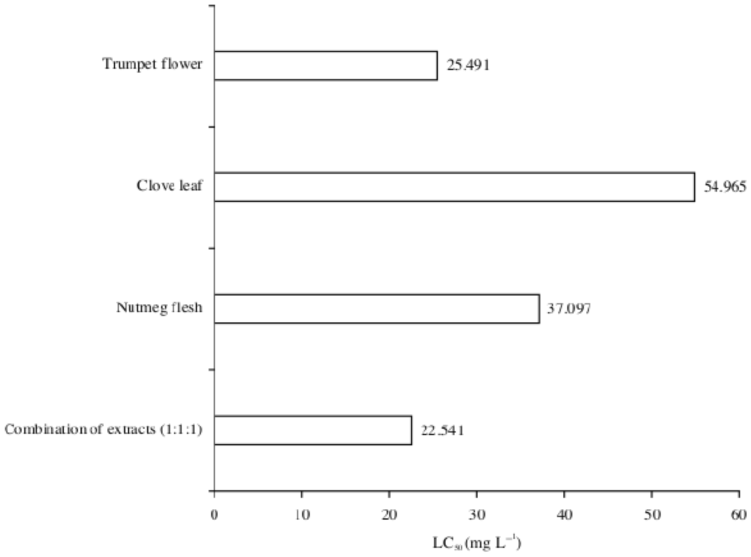

Fig. 3: Combination of extracts with a ratio of 1:1:1 showed the best larvicidal activity
y-axis: Variety of extract

DISCUSSION

Based on the percent yield, nutmeg pulp extract showed the highest yield percentage compared to clove leaf extract and trumpet flower extract. Ethanol solvent was effective in attracting secondary metabolites contained in nutmeg flesh. The results of the analysis of phytochemical groups found that the content of phenolic compounds was evenly distributed in the three types of extracts. However, there are variations in the content of steroids and triterpenoids in the three types of extracts.

Extraction of plant material is an essential step in obtaining secondary plant metabolites used as raw materials for drugs, cosmetics and insecticides. There are many methods for extracting plant material, including percolation, soxhletation and steam distillation methods[27,28]. The percolation method is only suitable for soluble organic compounds, while soxhletation is only good for heat-resistant compounds. Therefore, the maceration method was chosen to maximize the isolation of secondary metabolites from plant extracts.

Of the various plant extraction methods, maceration is the most widely used in extraction to develop drugs and insecticides[29,30]. Maceration was chosen because this Extraction provides time for the solvent to attract secondary metabolites in the Simplicia and is carried out at room temperature so that the compound is not damaged or its activity decreases due to exposure to high temperatures. The most important factors affecting the extraction results are solvent, plant samples, time and temperature[25,29].

Based on the content analysis of phytochemical groups, it was found that all phytochemical test groups were found in all types of extracts. Because Simplicia was extracted with a polar solvent, namely ethanol, the polar compounds were attracted quite well, indicated by the colour intensity of the high phytochemical content test. All extracts showed high levels of flavonoid content. However, after the extracts of nutmeg pulp, clove leaves and trumpet flowers were combined in a 1:1:1 (w/v), it resulted in high-intensity content of all phytochemical groups. This shows that the mixing of the three types of extracts increases the content of the phytochemical group.

Based on the results of quantitative analysis using the standard flavonoid quercetin, it also strengthens the results of the study of the content of qualitative phytochemical groups in the combination of extracts. The highest quercetin content was found in the combination of extracts compared to other partial extracts. Flavonoids and other phenolic compounds are widely reported to have pharmacologic and insecticidal activity.

The test concentration of 1000 ppm showed the highest larval mortality in all types of extracts and combinations of extracts. The highest LC_{50} was shown by trumpet flower extract, this indicates the highest toxicity level of trumpet flower extract to mosquito larvae. Furthermore, the lowest toxicity was based on the LC_{50} value of the nutmeg pulp extract. However, overall the best LC_{50} was shown in the combination of extracts. Thus, the combination of extracts increased the toxicity of mosquito larvae.

Observations on the toxicity test included larvae often appearing on the surface. The frequency was very long, which indicated that the need for oxygen dissolved in the water was reduced. The larvae often came to the surface to meet their oxygen needs, another symptom was a reduced response to stimuli marked by reduced aggressiveness when the test bottle was touched. This situation is an active symptom caused by the bioactive compounds in the test extract. The content of phytochemical groups was found evenly in all types of extracts. However, the phenolic compound group showed the highest intensity of content both in the extract of nutmeg flesh, trumpet flower, clove leaf and combination of extracts.

Several phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, saponins and steroids have been reported cytotoxic effects *in vitro*[31,32]. These compounds can enter the larval body, which has a thin cell membrane. Toxic compounds enter through several parts of the body, including the body's surface, the respiratory tract and the digestive tract. The body surface wall is the outermost part of the larva's body that can absorb large amounts of insecticide because this part is directly related to insecticides[33,34]. In larval respiration, air and oxygen enter the trachea by diffusion with the help of abdominal movements and toxic substances in the extract can also enter the respiratory system in the form of gas or fine granules carried to living tissue. In this study, toxic substances enter the mouth of the larvae through the respiratory system in the form of spiracles on the body's surface and cause wilting of the nerves and damage to the spiracles larvae cannot breathe and eventually die. One group of plant phytochemicals reported causing nerve wilting is saponins. Saponins can inhibit the action of the enzyme acetylcholinesterase. Acetylcholine formed by the central nervous system serves to deliver impulses from nerve cells to muscle cells. After the impulse is transmitted the enzyme acetylcholinesterase stops the process, which breaks down acetylcholine into acetyl Co-A and choline. The presence of insecticidal compounds (alkaloids, flavonoids and saponins) will inhibit the work of this enzyme resulting in a buildup of acetylcholine which will cause chaos in the impulse delivery system to muscles and can result in muscle spasms, paralysis and eventually death[35-37].

One of the mechanisms of flavonoid toxicity is also a strong respiratory inhibitor. Components that interfere with energy metabolism have been identified from either natural or synthetic sources. Energy metabolism disorders occur in the mitochondria by inhibiting the electron transport system or by blocking the coupling between the transport system and ATP production. Inhibition of the electron transport system blocks ATP production and causes a decrease in mitochondrial oxygen consumption. One of them is also a strong respiratory inhibitor. Respiratory inhibitors work by inhibiting the respiratory chain, inhibiting oxidative phosphorylation, or uncoupling the respiratory chain with oxidative phosphorylation. Electron transport inhibitors at the site I work by inhibiting Coenzyme Q reductase (NADH oxidase inhibitor). Inhibitor at site II by inhibiting Cytochrome b-c complex[38-41].

Impaired energy metabolism and loss of ATP cause slow-acting toxicity and affect all components, including paralysis and larval death. Kaempferol, Myricetin and Quercetin are included in one of the flavonoid groups, namely flavonols. Flavonoids are good reducing agents so that they can inhibit oxidation reactions, both enzymatically and non-enzymatically[42]. Flavonoids enzymatically inhibit the oxidation process by acting as ATPase inhibitors, NADH-oxidase-inhibitors (Coenzyme Q reductase inhibitors) and cytochrome inhibitors[43,44]. The presence of NADH-oxidase-inhibitor (Coenzyme Q reductase inhibitor) causes blockage of electron flow from NADH to CoQ (Coenzyme Q). Cytochrome inhibitors block the flow of electrons from cytochrome b to c1. By blocking the flow of electrons from cytochrome b to c1, all electron acceptors before cytochrome b are reduced. The ATPase inhibitors act at site V by inhibiting the ATP synthesis catalyst from ADP[45].

The larvicidal mechanism of tannins is related to their ability to inactivate adenosine, enzymes and cell proteins[46,47]. Hydrolyzed tannins are usually amorphous compounds, hygroscopic, yellow-brown in colour and soluble in water (boiling water) to form colloidal solutions instead of natural solutions. The purer the tannin, the less soluble it is in water and the easier it is to obtain it in crystalline form. The interaction of tannins with proteins is characteristic and depends on the structure of the tannins[48,25]. Some tannins have been shown to have an activity to inhibit reverse transcriptase and DNA topoisomerase enzymes[46]. Tannins bind to polysaccharides and are soluble in water. Catechin tannins play an essential role as larvicides because they cause damage to cell membranes so that mosquito larvae die. The part of flavonoid larvicides occurs through the mechanism of inhibition of nucleic acid (DNA) synthesis of larvae, which causes the death of the larvae[49].

The results of this study provide a new alternative to the use of ethnobotanical plants as a source of bioactive mosquito larvicides. However, vector control is more effective than disease control. Furthermore, larvae are a more sensitive stage for controlling mosquito populations. However, further research on larvicidal formulations and large-scale testing are needed to obtain the best formulation of ethnomedical plant larvicides.

CONCLUSION

The combination of clove leaf extract, nutmeg flesh extract and trumpet flower synergistically increases the bioactive content. Flavonoids increased in the combination of extracts compared to partial extracts. High phenolic compounds in all types of extracts were cytotoxic to mosquito larvae. The combination of extracts showed the highest toxicity to mosquito larvae (LC_{50}: 22.541 mg L^{-1}), while the lowest was a partial extract of clove leaves with LC_{50} (54.965 mg L^{-1}).

SIGNIFICANCE STATEMENT

This study strengthens the empirical and ethnomedical use of medicinal plants for mosquito control. North Sulawesi as a malaria-endemic area has ethnomedical medicinal plants as mosquito bioinsecticides which are passed down from generation to generation. However, this study scientifically proves the bioactive potential of the combination of ethnobotanical plant extracts as larvicides. This research opens the field of research on the use of bioactive medicinal plants for mosquito repellent materials, liquid mosquito repellents and other forms of mosquito control in the future.

ACKNOWLEDGMENTS

We want to thank the Directorate of Research and Community Service, Ministry of Education, Culture, Research and Higher Education, the Republic of Indonesia for funding this research through the Basic Research Scheme for Higher Education Excellence in 2021. This research was funded through a superior basic research scheme of higher education with decree number 129/E4.1/AK.04.PT/2021 and Contract Agreement Number 441/UN41.9/TU/2021. We thank the leadership and staff of the Laboratory of Bioactivity and Molecular Biology, State University of Manado and the Laboratory of PSB Biopharmaca IPB Bogor for assisting the analysis.

REFERENCES

1. Mbatchou, V.C., D.P. Tchouassi, R.A. Dickson, K. Annan and A.Y. Mensah *et al.*, 2017. Mosquito larvicidal activity of *Cassia tora* seed extract and its key anthraquinones aurantio-obtusin and obtusin. Parasites Vectors, Vol. 10. 10.1186/s13071-017-2512-y.
2. Anoopkumar, A.N. and E.M. Aneesh, 2022. A critical assessment of mosquito control and the influence of climate change on mosquito-borne disease epidemics. Environ. Dev. Sustainability, 24: 8900-8929.
3. Adam, A. and C. Jassoy, 2021. Epidemiology and laboratory diagnostics of dengue, yellow fever, zika, and chikungunya virus infections in Africa. Pathogens, Vol. 10. 10.3390/pathogens10101324.
4. Franklinos, L.H.V., K.E. Jones, D.W. Redding and I. Abubakar, 2019. The effect of global change on mosquito-borne disease. Lancet Infect. Dis., 19: E302-E312.
5. Deepak, P., R. Sowmiya, G. Balasubramani, D. Aiswarya, D. Arul, M.P.D. Josebin and P. Perumal, 2018. Mosquito-larvicidal efficacy of gold nanoparticles synthesized from the seaweed, *Turbinaria ornata* (Turner) J.Agardh 1848. Part. Sci. Technol., 36: 974-980.
6. Louis, M.R.L.M., V. Pushpa, K. Balakrishna and P. Ganesan, 2020. Mosquito larvicidal activity of avocado (*Persea americana* Mill.) unripe fruit peel methanolic extract against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi*. South Afr. J. Bot., 133: 1-4.
7. Haryanto, B., 2020. Indonesia: Country report on children's environmental health. Rev. Environ. Health, 35: 41-48.
8. Mordecai, E.A., S.J. Ryan, J.M. Caldwell, M.M. Shah and A.D. LaBeaud, 2020. Climate change could shift disease burden from malaria to arboviruses in Africa. Lancet Planet. Health, 4: e416-e423.
9. Harapan, H., A. Michie, M. Mudatsir, R.T. Sasmono and A. Imrie, 2019. Epidemiology of dengue hemorrhagic fever in Indonesia: Analysis of five decades data from the national disease surveillance. BMC Res. Notes, Vol. 12. 10.1186/s13104-019-4379-9.
10. Ramadhani, S.N. and M.T. Latif, 2021. Impact of climate change on dengue hemorrhagic fever (DHF) in tropical countries: A literature review. J. Kesehatan Lingkungan, 13: 219-226.
11. Sun, Z., M. Lv, W. Huang, T. Li and H. Xu, 2022. Development of botanical pesticides: Exploration on the phenotype of vestigial wings of insect pests induced by plant natural products or their derivatives by blocking tyrosine phosphorylation of insulin receptor 1. J. Agric. Food Chem., 70: 2117-2126.
12. Lindsay, S.W., M.B. Thomas and I. Kleinschmidt, 2021. Threats to the effectiveness of insecticide-treated bednets for malaria control: Thinking beyond insecticide resistance. Lancet Global Health, 9: e1325-e1331.

13. Taiwo, A.M., 2019. A review of environmental and health effects of organochlorine pesticide residues in Africa. Chemosphere, 220: 1126-1140.
14. Isman, M.B., 2020. Botanical insecticides in the twenty-first century-fulfilling their promise? Annu. Rev. Entomol., 65: 233-249.
15. Gajger, I.T. and S.A. Dar, 2021. Plant allelochemicals as sources of insecticides. Insects, Vol. 12. 10.3390/insects12030189.
16. Silvério, M.R.S., L.S. Espindola, N.P. Lopes and P.C. Vieira, 2020. Plant natural products for the control of *Aedes aegypti*: The main vector of important arboviruses. Molecules, Vol. 25. 10.3390/molecules25153484.
17. Carmona-Hernandez, S., J.J. Reyes-Pérez, R.G. Chiquito-Contreras, G. Rincon-Enriquez, C.R. Cerdan-Cabrera and L.G. Hernandez-Montiel, 2019. Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: A review. Agronomy, Vol. 9. 10.3390/agronomy9030121.
18. Iwuagwu, M.O., P.E. Etusim, N.C. Emmanuel, J.C. Igwe, V.O. Nwaugo and R.A. Onyeagba, 2020. Exploitation of plant herbs in the control of disease vectors: A review. Pharm. Biosci. J., 8: 7-21.
19. Wurarah, M. and Y.S. Mokosuli, 2022. Inhibition of bacterial growth of leilem leaf extract (*Clerodendrum minhassae* Teijsm. & Binn): Inhibition of bacterial growth of leilem leaf extract. J. Trop. Biol., 22: 549-556.
20. Barik, M., A. Rawani, S. Laskar and G. Chandra, 2019. Evaluation of mosquito larvicidal activity of fruit extracts of *Acacia auriculiformis* against the Japanese encephalitis vector *Culex vishnui*. Nat. Prod. Res., 33: 1682-1686.
21. Hari, I. and N. Mathew, 2018. Larvicidal activity of selected plant extracts and their combination against the mosquito vectors *Culex quinquefasciatus* and *Aedes aegypti*. Environ. Sci. Pollut. Res., 25: 9176-9185.
22. Oladipupo, S.O., A. Callaghan, G.J. Holloway and O.A. Gbaye, 2019. Variation in the susceptibility of *Anopheles gambiae* to botanicals across a metropolitan region of Nigeria. PLoS ONE, Vol. 14. 10.1371/journal.pone.0210440.
23. Sayono, S., R. Anwar and D. Sumanto, 2020. Larvicidal activity of ethyl acetate extract of *Derris elliptica* root against the third-instar larvae of cypermethrin-resistant *Aedes aegypti* offspring. J. Arthropod-Borne Dis., 14: 391-399.
24. Kaunang, E.N.S. and M.Y. Semuel, 2017. Botanical and phytochemical constituents of several medicinal plants from mount Klabat North Minahasa. J. Med. Plants Stud., 5: 29-35.
25. Semuel, M.Y., E.S.N. Kaunang and J.S. Manopo, 2019. The bioactive contents and antioxidant activity of honey bee nest extract of *Apis dorsata* Binghami from the North Sulawesi. Molekul, 14: 92-102.
26. Stanković, M.S., I.D. Radojević, O.D. Stefanović, M.D. Topuzović, L.R. Čomić and S.R. Branković, 2011. Immortelle (*Xeranthemum annuum* L.) as a natural source of biologically active substances. EXCLI J., 10: 230-239.
27. Tembo, Y., A.G. Mkindi, P.A. Mkenda, N. Mpumi and R. Mwanauta *et al*., 2018. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. Front. Plant Sci., Vol. 9. 10.3389/fpls.2018.01425.
28. Amoabeng, B.W., A.C. Johnson and G.M. Gurr, 2019. Natural enemy enhancement and botanical insecticide source: A review of dual use companion plants. Appl. Entomol. Zool., 54: 1-19.
29. Castillo-Henríquez, L., K. Alfaro-Aguilar, J. Ugalde-Álvarez, L. Vega-Fernández, G.M. de Oca-Vásquez and J.R. Vega-Baudrit, 2020. Green synthesis of gold and silver nanoparticles from plant extracts and their possible applications as antimicrobial agents in the agricultural area. Nanomaterials, Vol. 10. 10.3390/nano10091763.
30. Uwineza, P.A. and A. Waśkiewicz, 2020. Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. Molecules, Vol. 25. 10.3390/molecules25173847.
31. Hossain, F., M.G. Mostofa and A.H.M.K. Alam, 2021. Traditional uses and pharmacological activities of the genus leea and its phytochemicals: A review. Heliyon, Vol. 7. 10.1016/j.heliyon.2021.e06222.
32. Batiha, G.E.S., A.M. Beshbishy, O.S. Adeyemi, E.H. Nadwa and E.K.M. Rashwan *et al*., 2020. Phytochemical screening and antiprotozoal effects of the methanolic *Berberis vulgaris* and acetonic *Rhus coriaria* extracts. Molecules, Vol. 25. 10.3390/molecules25030550.
33. Subahar, R., A. Aulung, I. Husna, R. Winita, L. Susanto, N.S. Lubis and N.E. Firmansyah, 2020. Effects of *Lansium domesticum* leaf extract on mortality, morphology, and histopathology of *Aedes aegypti* larvae (Diptera: Culicidae). Int. J. Mosq. Res., 7: 105-111.
34. Maula, L.N., Martini and M.S. Adi, 2021. Papaya leaves extract effectiveness test (*Carica papaya* L) as a larvacidine *Aedes aegypti* instar III. Int. J. Health Educ. Social, 4: 20-29.
35. de Oliveira, T.S., D.V. Thomaz, H.F. da Silva Neri, L.B. Cerqueira and L.F. Garcia *et al*., 2018. Neuroprotective effect of *Caryocar brasiliense* Camb. leaves is associated with anticholinesterase and antioxidant properties. Oxid. Med. Cell. Longevity, Vol. 2018. 10.1155/2018/9842908.
36. Zhang, L., G. Zengin, G. Rocchetti, I. Senkardes and J.B. Sharmeen *et al*., 2021. Phytochemical constituents and biological activities of the unexplored plant *Rhinanthus angustifolius* subsp. grandiflorus. Appl. Sci., Vol. 11. 10.3390/app11199162.
37. Oni, M.O., O.C. Ogungbite, S.O. Oguntuase, O.S. Bamidele and T.I. Ofuya, 2019. Inhibitory effects of oil extract of green acalypha (*Acalypha wilkesiana*) on antioxidant and neurotransmitter enzymes in *Callosobruchus maculatus*. J. Basic Appl. Zool., Vol. 80. 10.1186/s41936-019-0116-0.

38. Gupta, P.K., 2019. Toxic Effects of Pesticides and Agrochemicals. In: Concepts and Applications in Veterinary Toxicology, Gupta, P.K. (Ed.), Springer, Cham, Switzerland, ISBN: 978-3-030-22252-9, pp: 59-82.
39. Fernandes, D.A., R.P.C. Barros, Y.C.F. Teles, L.H.G. Oliveira and J.B. Lima *et al.*, 2019. Larvicidal compounds extracted from *Helicteres velutina* K. schum (sterculiaceae) evaluated against *Aedes aegypti* L. Molecules, Vol. 24. 10.3390/molecules24122315.
40. Ferreira, M.D.L., D.A. Fernandes, F.C. Nunes, Y.C.F. Teles and Y.M. Rolim *et al.*, 2019. Phytochemical study of *Waltheria viscosissima* and evaluation of its larvicidal activity against *Aedes aegypti*. Rev. Bras. Farmacognosia, 29: 582-590.
41. Karthi, S., K. Uthirarajan, V. Manohar, M. Venkatesan, K. Chinnaperumal, P. Vasantha-Srinivasan and P. Krutmuang, 2020. Larvicidal enzyme inhibition and repellent activity of red mangrove *Rhizophora mucronata* (Lam.) leaf extracts and their biomolecules against three medically challenging arthropod vectors. Molecules, Vol. 25. 10.3390/molecules25173844.
42. Emam, M., D.R. Abdel-Haleem, M.M. Salem, L.J.M. Abdel-Hafez and R.R.A. Latif *et al.*, 2021. Phytochemical profiling of *Lavandula coronopifolia* poir. Aerial parts extract and its larvicidal, antibacterial, and antibiofilm activity against *Pseudomonas aeruginosa*. Molecules, Vol. 26. 10.3390/molecules26061710.
43. Puri, S., S. Singh and S.K. Sohal, 2021. Growth retarding effect of curcumin on *Bactrocera cucurbitae* (Coquillett) larvae. Arch. Phytopathol. Plant Prot., 54: 722-735.
44. Zulhussnain, M., M.K. Zahoor, H. Rizvi, M.A. Zahoor and A. Rasul *et al.*, 2020. Insecticidal and genotoxic effects of some indigenous plant extracts in *Culex quinquefasciatus* say mosquitoes. Sci. Rep., Vol. 10. 10.1038/s41598-020-63815-w.
45. Matiadis, D., P.G.V. Liggri, E. Kritsi, N. Tzioumaki and P. Zoumpoulakis *et al.*, 2021. Curcumin derivatives as potential mosquito larvicidal agents against two mosquito vectors, *Culex pipiens* and *Aedes albopictus*. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22168915.
46. Redo, T., T. Triwani, C. Anwar and S. Salni, 2019. Larvicidal activity of ketapang leaf fraction (*Terminalia catappa* L) on *Aedes aegypti* instar III. Open Access Maced. J. Med. Sci., 7: 3526-3529.
47. Soni, N. and R.C. Dhiman, 2020. Larvicidal and antibacterial activity of aqueous leaf extract of peepal (*Ficus religiosa*) synthesized nanoparticles. Parasite Epidemiol. Control, Vol. 11. 10.1016/j.parepi.2020.e00166.
48. Ravindran, D.R., M. Bharathithasan, P. Ramaiah, M.S.M. Rasat and D. Rajendran *et al.*, 2020. Chemical composition and larvicidal activity of flower extracts from *Clitoria ternatea* against *Aedes* (Diptera: Culicidae). J. Chem., Vol. 2020. 10.1155/2020/3837207.
49. Karthiga, P., S. Rajeshkumar and G. Annadurai, 2018. Mechanism of larvicidal activity of antimicrobial silver nanoparticles synthesized using *Garcinia mangostana* bark extract. J. Cluster Sci., 29: 1233-1241.

Mosquito Larvicides of Partial and Combinations Extract of Ethnobotanical Plant from North Sulawesi, Indonesia

ORIGINALITY REPORT

3%	3%	6%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

3%

★ Khadijah, Nunuk Hariani Soekamto, Siti Maisuri Tadjuddin Chalid, Nur Fatin Rafidah. " Total Phenol Content and Activities of Antioxidant Extracts Methanol Limes By Uv-Vis Spectrophotometry ", E3S Web of Conferences, 2021

Publication

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		