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Larvicidal And Repellent Potential Of Patchouli Extract (*Pogostemon Cablin*) Varieties Of Southeast Sulawesi For *Aedes Aegypti* Vector

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Abstract

The development of resistance to chemical insecticides has become a problem in vector control. This demands the need for research and development of environmentally friendly methods for vector control of *Aedes aegypti*. The efficacy of *Pogostemon cablin* extract as a repellent for adult mosquitoes is an alternative. Repellent can reduce exposure to mosquito bites that may be infected with the dengue virus. To conduct phytochemical testing and content analysis of essential oil of Patchouli (*Pogostemon cablin*) varieties of Southeast Sulawesi. Research samples were the leaves and stems of the patchouli plant (*Pogostemon cablin*) of the Southeast Sulawesi variety. This research uses the Harbone method of phytochemical testing and essential oil distillation. The results of the phytochemical test of patchouli leaves (*Pogostemon cablin*) of Southeast Sulawesi varieties were alkaloids, flavonoids, triterpenoids, polyphenols and terpenoids, while patchouli stems contained alkaloids, triterpenoids, tannins, polyphenols and terpenoids. The test results for the essential oil content of the patchouli leaves identified eugenol, patchouli alcohol, linalool and a-pinene. The leaves and stems of patchouli plant have potential as larvicides and repellents for the vector of Dengue Hemorrhagic Fever, *Aedes aegypti*.

Keywords: *Aedes aegypti*; *Pogostemon cablin*; phytochemical; essential oil; Dengue Hemorrhagic Fever

1. Introduction

Dengue Hemorrhagic Fever (DHF) is caused by the dengue virus which is transmitted by the *Aedes sp.* Mosquito. This vector is anthropophilic, lives close to humans and is often indoors. Vector control by chemical means is currently widely applied. One of the application methods called fogging has been reported to be less effective in killing targets and contributes to increasing vector resistance to insecticides[1–4].

Eco-friendly efforts to prevent the transmission of the dengue virus as the cause of DHF can be done by using plant-derived insecticides, both for adult mosquitoes, larvae and as protection against mosquito bites (*repellent*). Repellent is a substance that acts locally or at a certain distance to prevent insects from flying, landing or biting the skin of humans and animals[3,5]. Repellent can reduce exposure to mosquito bites that may be infected with the dengue virus[6,7]

Currently DEET (N,N Dimethyl-meta-toluamide) is the main insect repellent. However, its use causes many disadvantages such as the level of toxicity to the skin and also has an negative impact on the central nervous system, if its application is not done properly[8]. Considering the dangers of using synthetic repellents, it is necessary to replace them. This is because the chemical which is widely traded as a base material for synthesizing repellents contains halogenated hydrocarbons which are known to have

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relatively long half-lives to decompose and are feared for their toxic properties[9]. If these chemical compounds accumulate in the body or the concentration exceeds the body's tolerance threshold, it will have a detrimental effect on health [10].

Repellent works by protecting the skin when applied, preventing bites or contact with mosquitoes[11,12]. There are many health benefits derived from repellent applications, especially protection against vector bites such as *Aedes aegypti*. In addition, the use of repellents is very practical and economical[13]. One of the plants that has the potential as a repellent is patchouli. It was also reported that the patchouli plant contains tannins, flavonoids and terpenoids [14]. Previous studies have stated that the content of essential oils in plants, when applied as a repellent, has potential protective power against *Aedes sp*[15–19]. The mechanism of action of repellents is to confuse or interfere with the olfactory senses of mosquitoes in detecting chemical compounds produced by humans and mammals, thereby protecting repellent users from mosquitoes by preventing their landing and biting[20–23].

The development of repellents from plants has been reported in several countries, including Indonesia. Research shows that several plants in Indonesia such as betel (*Piper betle*), lemongrass (*Cymbopogon citrates*), basil (*Ocimum spp.*) and kaffir lime leaves have potential as repellent[24–26]. In Thailand, it was reported that *Zanthoxylum piperitum*, *Anethum graveolens* and *Kaempferia galanga* plants have potential as repellents because they have more than 50% repellency against *Aedes aegypti* mosquitoes[8]. Previous studies reported that several plants from the Lamiaceae, Labiateae, Rutaceae, and Mirtaceae families have repellent activity against *Aedes aegypti*[11].

Research on the use of patchouli plants as lotions for mosquito repellent substances (repellent) needs to be conducted in order to determine the potential of patchouli plants of Southeast Sulawesi varieties whose contents have not been studied and their effects as mosquito repellent. This is because there are various factors that cause differences in patchouli oil production such as climatic characteristics and land characteristics, including altitude, slope, conditions of small rocks above the land surface, and others. In addition, according to Nickavar et al. (2004), differences in the composition and amount of components that make up the oil can be caused by the variability of different plant subspecies[27]. From the description above, it is important to conduct research through the phytochemical and content analysis of essential oil of Patchouli (*Pogostemon cablin*) varieties of Southeast Sulawesi.

This study aimed to conduct the phytochemical and content analysis of the essential oil of patchouli (*Pogostemon cablin*) varieties of Southeast Sulawesi

2. Experiment

a. Research Time and Place

The research was conducted from March to December in 2021. The research sites were at the Chemistry Laboratory of Haluoleo University and the Parasitology Laboratory of the Health Polytechnic of the Ministry of Health Kendari.

b. Sample preparation and extraction

Patchouli plant sampling was done in the Konawe Village, Wawotobi District, Konawe Regency, Southeast Sulawesi. The leaves and stems of the patchouli plant were washed then dried and crushed to form a powder. Next, preparations were made for the isolation of essential oils.

c. Equipment

Tools: Digital scale, pH meter, dropper, measuring pipette, treatment cup, measuring cup, test tube, glass stir bar, funnel, label paper, and Rotary vacuum evaporator. **Ingredients:** Patchouli, methanol, ethanol, NaOH, H_2SO_4, Zn powder, Mayer reagent, HCl, and FeCl 1%

d. Preparation of Patchouli leaf simplicia (*Pogostemon cablin*) and preparation of patchouli leaf extraction

Patchouli leaf simplicia and patchouli stems (*Pogostemon cablin*) were separated, then collected and washed under running water, drained and then chopped. Samples were dried by drying in the open air protected from sun exposure. Then, the sample was sieved using a 60 mesh sieve. The sample that passed the sieve was put into a beaker and methanol was added (1:4) as solvent. The samples were soaked for 5 days and homogenized by stirring occasionally. Then, the liquid extract was filtered using filter paper and the filtrate was collected. The sample was continued by maceration for 2 days with new methanol solvent. The filtrate obtained was concentrated with a rotary vacuum evaporator to remove the solvent.

e. Determination of Chemical Composition

Determination of the chemical composition of patchouli was done through a qualitative test using the Harborne (1973),Trease and Evans (1989) and Sofowora (1993) methods. Determination of the chemical composition of patchouli oil was carried out by gas chromatography, type HP 7890 combined with mass spectroscopy type HP 5975B with ionization by electron collision (70 eV), equipped with a capillary column HP-innowax 30 x 0.25 mm, film thickness 0.5 m. The oven temperature was programmed at 60-150°Cat 2°C/min and then 150-210°C and maintained at 210°C for 10 minutes. The carrier gas was helium, the injector temperature was 230°C, at a flow rate of 0.6 mL/min with a split ratio of 1:250.

Identification of Flavonoids

A total of 0.1 g of thick extract was dissolved in 10 mL of ethanol then divided into four test tubes. The first tube was used as a positive control, the second tube contained the sample plus NaOH, the third tube contained the sample plus concentrated H_2SO_4, and the fourth tube contains the sample plus Zn powder. Color changes that occurred in the second, third and fourth tubes were observed and compared with positive control tubes. If there is a color change, then the sample is positive for flavonoids.

Identification of Alkaloids

Into 0.5 g of thick extract, 2 mL of 70% ethanol was added and then stirred. The mixture is filtered and a little hot water is added to the filtrate. After cooling, the mixture was filtered and 2-3 drops of Mayer's reagent were added to the filtrate. If the sample becomes cloudy or a precipitate forms, it indicates a positive sample containing alkaloids.

Identification of Saponins

A total of 0.1 g of thick extract was dissolved in 15 mL of hot water and heated for 5 minutes. The mixture was filtered and the filtrate was put into a test tube and shaken until frothy, then 2N HCl was added. The sample is said to be positive for saponins if the foam / foam persists for 10 minutes.

Identification of Tannins

A total of 0.1 g of thick extract was dissolved in methanol, then 2-3 drops of 1% $FeCl_3$ solution were added. The sample is tested positive for tannin if a yellow precipitate is formed.

Phenolic Identification

A total of 0.1 g of thick extract was added to 20 mL of FeCl3 solution. A positive test for the presence of phenolics is the formation of a green to blue-black color.

Terpenoid Test

The test was done by taking 2 mL each. Patchouli leaf and stem samples were extracted with water and ethanol as solvents. After that, 3 drops of concentrated HCl and 1 drop of concentrated $H_2SO_4$was added to each extract. If each solution is colored or purple, then it is positive that it contains terpenoids.

Steroid Test

The test was done by taking 2 mL of patchouli plant samples that had been extracted with water and ethanol as solvents. After that, 3 drops of concentrated HCl and 1 drop of concentrated H_2SO4 was added to each extract. If each solution forms a green color, it is positive that it contains steroids..

f. Essential Oil Insulation

Distillation procedure of local patchouli essential oil (*Pogostemon cablin*)

A total of 75 grams of dried patchouli leaves were cut into small pieces using a blender and put into a 500 mL round bottom flask. Enough aquadest was added until sample was submerged, Next, the flask was connected to a distiller and simmered for 4-5 hours at 100^oC to produce oil. The distillation ends when a yellow or brownish yellow color comes out which is the color of patchouli oil. The distillate obtained was accommodated into an Erlenmeyer beaker. The distillate was then added with $CaCl_2$ to bind the water which was still mixed with patchouli oil.

Physical properties check

Examination of the physical properties of the volatile oil components of patchouli batik leaves includes examination of color, examination of odor, solubility in alcohol, determination of specific gravity and determination of refractive index. Patchouli leaf essential oil examination was done with the parameters of smell, color, and taste. The refractive index examination was conducted using the Abbe Refractometer ATAGO at a temperature of 22o C.

Patchouli leaf essential oil was dropped on the main prism, then the prism was closed and the refractometer was directed to the bright light through a scale lens so that it could be seen clearly. Furthermore, the value of the refractive index is indicated by the boundary line that separates the bright side and the dark side at the top and bottom which can be seen through a microscope.

Physicochemical properties

By using the standard procedure of the Indonesian Herbal Pharmacopoeia (FHI),the physicochemical properties of patchouli oil were determined and compared with standard specifications. The measurement of physicochemical properties of the oil includes specific gravity, refractive index, optical rotation, solubility in alcohol, and determination of patchouli alcohol content. All these parameters use the 2006 Indonesian National Standard (SNI 06-2385-2006).

Determination of chemical composition

The chemical component test of patchouli oil was conducted by gas chromatography, type HP 7890 coupled with mass spectroscopy, type HP 5975B with electron impact ionization (70 eV), equipped with an HP-innowax 30 x 0.25 mm capillary column, film thickness 0.5 m. The oven temperature was programmed at 60-150°C at 2°C/min and then 150-210°C and maintained at 210°C for 10 minutes. The carrier gas was helium, the injector temperature was 230°C, at a flow rate of 0.6 mL/min with a split ratio of 1:250. The essential oil was injected automatically via Split Mode. The device is controlled by a computer system that manages mass spectrum data and is compared with published standard data

3. Results

In this study, tests were done to determine the chemical content, namely qualitative methods and distillation methods. The test method to determine the content of secondary metabolites uses a qualitative method that refers to the Harborne method. The content of secondary metabolites was obtained through a qualitative test on patchouli leaves using methanol as a solvent.

a. Results of the Phytochemical test

In Table 1, the results of the Harbone method test for both the leaves and stems of patchouli identified alkaloids, triterpenoids, polyphenols and terpenoids. Meanwhile, flavonoids were only found in the leaves and specifically, tannins were only found in patchouli stems

Table 1. The results of the qualitative test of patchouli leaves and patchouli stems using the Harmonie method

Parameters	standard indicators	Patchouli Leaf	Patchouli Stem
Alkaloid :			
a. Dragendrof	Formation of an orange or red precipitate	+	+
b. Meyer	A slightly yellowish or white precipitate is formed	+	+
c. Wagner	A reddish-brown precipitate is formed	+	+
Flavonoid	Changes green to orange or yellow, red	+	-
Saponin	Stable foam is formed	-	-
Triterpenoid	Formation of red color or there is a brown ring	+	+
Steroid	A greenish blue color is formed	-	-
Tannin (Gelatin 1%)	A white precipitate is formed	-	+
Polyphenol (FeCl 1%)	Formed dark brown	+	+
Terpenoid	A brown layer is formed	+	+

Description: + = identified; - = not identified

Table 2. The results of the essential oil yield test on patchouli leaves and patchouli stems

Patchouli oil immersion value	Volume	Patchouli oil immersion value	Volume
Patchouli Leaves	Distillate 1 (260 ml)	Patchouli Stem	Distillate 1 (250 ml)
	Distillate I1 (280 ml)		Distillate I1 (275 ml)
	Total distillate (540 mL)		Total distillate (525 mL)
	Dried distillate $CaCl_2$ (104 mL)		Dried distillate $CaCl_2$ (110 mL)
	Oil yield (19.11%)		Oil yield (20.95%)

Table 3. Characteristics of patchouli oil of Southeast Sulawesi varieties

Test	patchouli leaf	Patchouli stem	INS value
Organoleptic test	bright yellow	Light yellow brown	yellow
Specific gravity	0.954 g/ml	0.956 g/ml	0.943-0.983 g/ml
Sample weight	49.6882	49.7502	-
Refractive index	1.5068	1.5073	1.5040-1.5140
Empty pycno weight	28.2935	28.4451	-
Empty pycno weight + sample	77.9817	78.2053	-

Table 4. Density and viscosity values of Southeast Sulawesi patchouli

Sample	Temperature (°C)	Volume (mL)	Massa (g)	Flow time (s)	density (m/V)	viscosity (cP)
Water	29	50.7158	50.5974	6.5	0.9977	0.899
stem	29	50.7158	49.7502	9.71	0.9810	1.3205
Leaf	29	50.7158	49.6882	11.6	0.9797	1.5755

Table 5. Results of the chemical composition of patchouli leaf essential oil

Component	Area (%)
4 (Hexyloxy) benzoic acid	14. 87
2,4-Azetidione,3,3 diethyl-1 methyl	17.24
2,4 –Azetidion,3,3-diethyl	18.09
2,4 –Azetidione,3,3diethyl	18.37
N-(ter-Butyl)-1-cyclopropyl-2-methyl-5-Oxopyrrolidine-2-carboxamide	20.05
2,4-Azetidione,3,3-diethyl-1 methyl	21.94
N-(ter-Butyl)-1cyclopropyl-2-methyl-5-oxopyrrolidine-2-carboxamide	23.19
2,4-Azetidione,3,3 diethyl-1-methyl	27.66
N-(ter-Butyl)1-cyclopropyl-2-methyl-5-oxopyrrolidine-2-carboxamide	30.36

b. Essential Oil Content Test

The essential oil content test was conducted by the distillation method. The essential oil distillation method for repellant testing was done because the chemical content contained in essential oils greatly affects the protection against mosquitoes

Table 2 shows the results of the calculation of the yield of essential oil for patchouli leaves are 19.11% and the value of patchouli stem yield is 20.95%. This shows that the value of soaking leaves and stems has almost the same value.

Table 3 shows that for the organoleptic test, patchouli leaves werebright yellow and stems had light yellowish brown. The specific gravity for patchouli leaves is 0.954 and for patchouli stems is 0.956. The refractive indices of patchouli leaves and patchouli stems werenot much different. The results of organoleptic testing, specific gravity and refractive index were all in accordance with the criteria of the Indonesian National Standard (INS).

Table 4 shows the density and viscosity values of patchouli leaves are relatively higher than patchouli stems. The higher density and viscosity of patchouli leaves gives the potential for higher chemical content in it.

Testing the properties of the chemical composition of patchouli leaves was conducted in order to determine the essential oil content using a Gas Chromatography (GC-MS) tool. In this study, the HP-innowax column was used (Table 5). The main component of the leaf oil was N-(ter-Butyl)1-cyclopropyl-2-methyl-5-oxopyrrolidine-2-carboxamide (30.36%) and the lowest value was 4 (Hexyloxy) benzoic acid (Table 5)

4. Discussion

a. Phytochemical Analysis

The results of the phytochemical analysis in Table 1 show that the patchouli leaf extract contains positive alkaloids, flavonoids, triterpenoids, polyphenols and terpenoids. Meanwhile, the positive patchouli stem extract contains alkaloids, triterpenoids, tannins, polyphenols and terpenoids. The results of phytochemical screening indicate that it hasthe potential to become an insecticide[28]. This is in line with previous research that identified bioactive compounds such as flavonoids, phenolics, alkaloids, and terpenoids in plants have a repelling effect on mosquitoes[24,25,29,30]. The results showed that both the leaves and stems contained terpenoids and triterpenoids. Terpenoid compounds are reported to have high repellent activity against *Aedes aegypti*[31].

The content of flavonoids serves as a respiratory inhibitor. Flavonoids enter the body of the larvae through the respiratory system which will then cause withering of the nerves and damage to the respiratory system which causes the larvae to be unable to breathe, so the mosquito will die. In addition, flavonoids can also inhibit nucleic acid (DNA) synthesis. If DNA synthesis is inhibited, larval growth is not optimal and can even cause larvae to die, because DNA is needed for protein synthesis, where protein is needed for the growth and development process of larvae [32].

Alkaloids work by inhibiting the action of the acetylcholinesterase enzyme which plays a very important role in the nervous system This can cause disruption of acetylcholine degradation resulting in the accumulation of acetylcholine in the synaptic cleft. This situation can cause decreased muscle coordination, convulsions, respiratory problems, and death due to disruption of nerve impulse transmission. In addition, alkaloids also have a mechanism as a stomach poisoning agent[32].

Saponins are compounds that have soap-like properties, andcan remove the waxy coating on the outer membrane of the larva (cuticle) so that it can damage the layer. Saponins can also change the protein and lipid structure of cell membranes.These structural changes can cause a decrease in surface tension and intracellular osmosis, resulting in cell lysis.

Research conducted in Thailand [13] stated that the repellent activity of plants against several species of mosquitoes was also made possible by the synergistic effect of the combination of phytochemical content and essential oils [13] Regarding the repellent effect of plants against mosquito species in Thailand, it is known that the minimum time of protection against mosquitoes is 2 hours where the exposure time is 3 minutes.

b. Essential Oil Analysis

Several monoterpenes such as pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are the most abundant components in essential oils. These chemical components are reported to have repellent activity against mosquitoes [33–36]. Secondary metabolite components that have stronger protection against *Aedes aegypti* are sesquiterpenes and b-caryophyllene [31]. Although some repellent activity comes from several essential oils, what is often found is the presence of monoterpenes and sesquiterpenes [34,37]. Another research in Kenya[38] found that phytol, which is a diterpene alcohol derivative, had high activity against *Anopheles gambiae*.

The essential oil content produced by *Pogostemon cablin* is different from that reported by previous research[39,40]. The essential oil from the samples of *Pogostemon cablin*,which were collected from the Southeast Sulawesi variety, was shown to have varying amounts of the main compound. These changes in essential oil composition may arise from several environmental (climatic, seasonal, or geographic) and genetic differences. In this study, the specimens analyzed were collected from the Southeast Sulawesi region, which is a completely different biome when compared to other regions. Another study revealed that the factors that cause differences in patchouli oil production such as climatic properties and land characteristics include altitude, slope, conditions of small rocks above the land surface, and others. In addition, according to Nickavar et al.in 2004, differences in the composition and amount of components that make up the oil can be caused by the variability of different plant subspecies[27].

One of the factors that affect the extraction of bioactive components from plants is the extraction solvent. Since the components to be obtained are organic compounds, polar/apolar organic solvents are generally chosen for this study. Several studies previously reported on various phytochemicals extracted from plants that exhibit oviposition-preventing and ovicidal properties against *Aedes aegypti* or *Aedes albopictus* mosquitoes [41–44]. The oviposition area of the female mosquito is selected based on the olfactory, visual and tactical responses of the female individual [45].

In Ahbirami's study in 2014, ovicidal activity showed failure of hatchability of *Aedes albopictus* that prefer to lay eggs on moist surfaces or on water. Eggs can live until contact with water. The effect of the ovicidal compound depends on the penetration of the eggshell [46]. Our research revealed that patchouli (*Pogostemon cablin*) varieties of Southeast Sulawesi have potential as ovicidal agents and repellent because they contain alkaloids, flavonoids, triterpenoids, tannins, polyphenols and terpenoids. Research conducted by Fasomkusolsil et al. in 2012 reported the ovicidal effect of herbal essential oils on *Aedes aegypti*, *Anophelesdirus* and *Culex Quinquefasciatus* [43]. They also noted that dosage affects potency levels. A study by Govindjajar et al. in 2011 reported ovicidal activity for *Ocimum basilicum* on *Aedesageypti*, *Anopheles stephensi* and *Culex quinquefasciatus*[47]. This ovicidal potency appears to be a combination of the terpenoid, phenolic and tannin odors or the effect of the alkaloids in the plant content. Many studies report that the relationship between dose and time differs depending on the mosquito species [42,48]. Research conducted by Prajapati et al. in 2005 reported ovicidal prevention/oviposition and skin repellent activity of 10 different plant essential oils against *Anopheles. stepheni, Aedes aegypti and Culex quinquefasciatus*[41].

5. Conclusions

This study provides conclusive evidence of the repellent potential for *Pogestemon cablin* plants on *Aedes* spp. The results of this study also provide an opportunity to improve biological-based control products for *Aedes* spp. vector originating from the Southeast Sulawesi variety. These more ecofriendly options can contribute to reducing the high use of chemical insecticides and the resulting environmental pollution and health problems.

6. Acknowledment

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7. Conflicts of interest

The authors claim that they've no recognised competing economic hobbies or private relationships that could have appeared to persuade the paintings stated on this paper**.**

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