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Original Research Article

Comparison analysis of leaf and flower extraction of clove which have the potential as larvacida

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Efforts to control Dengue Fever both biologically, chemically and behaviourally continue to be pursued. One of the effects of chemical control is resistance to mosquitoes and environmental pollution. Alternative efforts by using larvicide from natural ingredients, one of which is derived from the clove plant. Cloves contain eugenol, saponins, flavonoids and tannins that can damage the nervous system of insects, interfere with food absorption and reduce digestive enzymes in insects. This study was to analyze the yield of clove flower and leaf extracts as an alternative to *Ae. aegypti* larvicides. This research is a laboratory experiment using a variety of methods and solvent variations. The results obtained were the yield of leaf extracts and flowers containing flavonoids, saponins, tannins which were the most larvicidal compounds found in leaves with a reflux process with Ethyl acetate solvent, which was 20.36%. The highest levels of flavonoids were found in the leaves through a reflux process with 96% Ethanol solvent levels of 29.53%. The highest levels of saponins were found in the leaves through the Soxhlet process with 96% ethanol as solvent, 9.92%. Clove flowers and leaves containing flavonoids and saponins can be used as an alternative as larvicide.

Keyword: Larvicide, clove leaves and flowers, control of Ae. aegypti larvae, flavonoid, saponin

INTRODUCTION

Dengue Hemorrhagic Fever is very well developed in Indonesia, which has a tropical climate. Dengue Hemorrhagic Fever is caused by viral infection of the *Flaviviridae* species, namely the *Flavivirus* genus with DEN-1, DEN-2, DEN-3, and DEN-4 serotypes (Irwadi et al., 2018). This environmental-based disease occurs due to urban development, climate change, increased mobility of population density, and low awareness of maintaining environmental cleanliness (Pradana et al., 2016).

The Indonesian Ministry of Health stated that the 3M Plus Mosquito Nest Eradication (PSN) consists of 3M, namely draining water reservoirs (TPA), such as bathtubs/WCs, drums and others at least once a week, closing TPA meetings such as water barrels/jars and so on, reuse used goods that can hold water and have the potential to be a breeding ground for *Ae. aegypti*. Meaning Plus itself means changing flower vase water, bird drinks, repairing damaged drains and gutters, cleaning places that can hold water such as banana midribs, and yards and gardens, keeping betta fish, tin head fish, using mosquito repellent, applying larvicide, using ovitrap, larvitrap, and mosquito traps and using mosquito repellent plants such as lavender, semar

bag, lemongrass, zodiac, geranium and others (Kementerian, 2016).

The use of chemical-based mosquito repellents has entered a dangerous stage, namely the presence of resistance in adult mosquitoes. Aedes aegypti in North Sumatra and Jambi provinces was resistant to *malathion* 0.8%, *cypermethrin* 0.05%, and *lambda cyhalothrin* and was still tolerant to alpha cypermethrin. Almost all strains of *Ae. aegypti* from North Sumatra and Jambi Provinces were resistant to 0.025% deltamethrin, only strains from Deli Serdang Regency were still tolerant to 0.025% *deltamethrin* (Sunaryo, 2018).

In line with the results of Heni's research (2016), which states that the use of *malathion* and t*emephos* in the long term causes resistance to *Ae. aegypti*. So it is necessary to replace insecticides with active ingredients that have been tested for their effectiveness and to increase efforts to eradicate mosquito nests in the community (Prasetyowati et al., 2016). Alternative efforts to eradicate mosquito nests through larval control are an option that is considered effective, safe for health and the environment. Biolarvicides are the right choice considering that there are many materials in nature that can be used as a reference for making biolarvicides. Several studies of biolarvicides have been carried out and are very useful for the development of larval control.

Based on the mortality rate of Thongpoon's research, the concentration at 500 ppm of extract showed the highest effectiveness in controlling the larvae with 98% mortality after exposure for 24 hr. This study suggests that *Millingtonia hortensis* L.f. extract can be used for controlling mosquito larvae especially *Aedes aegypti* Linn (Thongpoon and Poolprasert, 2015). The results of the Sapulette research (2019) showed that there was a larvicidal effect of steeping clove leaves (*Syzygium aromaticum* L.) on the mortality of *Anopheles* sp mosquito larvae, where the effective concentration was 6% and had an LC_{50} value in killing *Anopheles* sp larvae of 0.89%. The overall phytochemical content of clove leaves which has a fast response to the mortality of *Anopheles* sp larvae. are saponins, flavonoids and tannins that are able to act as larvicides through the mechanism of damaging cell membranes or disrupting larval metabolic processes (Sapulette et al., 2019).

Based on research conducted by Ekawati et al. (2017), it can be concluded that lime peel extract (*Citrus aurantifolia*) has the ability to kill Aedes aegypti mosquito larvae instar III, the effective concentration of lime peel extract (Citrus aurantifolia) which can kill 50% (IC50) *Aedes aegypti* mosquito larvae is 3.419% within 24 hours (Ekawati, Santoso, & Purwanti, 2017). The ethanol extract of citronella (Cymbopogon nardus L) which was researched by Arcani turned out to have potential as a natural larvicide of Ae. aegypti because it caused significant larval death in the treatment group. Concentrations of 0.05%, 0.1%, 0.2%, 0.5%, 1%, and 2% in the treatment group were declared effective as larvicides when compared to the control group with $p < 0.05$ (Arcani et al., 2017). Based on Sutiningsih research, Bruceine A isolated from the seeds of Brucea javanica (L.) Merr. has been demonstrated as biolarvicide against larvae of *A. aegypti* (L.), through its neurotoxic properties. The mechanism of neurotoxicity is mediated through inhibition of enzyme acetylcholinesterase and VGSC gene (Sutiningsih et al., 2017).

Based on Pamungkas research (2017) it was found that clove leaf essential oil of the *Zanzibar* variety had a larvicidal effect on *Ae. aegypti*. When compared with temephos as the gold standard for larvicides (WHO recommendation), at a concentration of 100 ppm clove leaf essential oil of the Zanzibar variety has the same effect as temephos (Pamungkas & Syafei, Neneng Syarifah; Soeroto, 2017). Sakriani et al. (2016) resulted in a study that the number of larvae that died on clove leaf juice was more than the number of larvae that died on papaya leaf juice in each concentration. Clove leaves and papaya leaves each contain compounds that can cause death in larvae. However, in this study, the number of larvae that died in a solution containing clove leaf juice with concentrations of 10%, 15%, and 20% was much higher than in a solution containing papaya leaf juice (Sakriani et al., 2016).

Considering the great potential of saponins and flavonoids as alternative biolarvicides found in clove leaves and flowers, it is necessary to conduct research on the most appropriate extraction method and solvent to obtain the percentage of extract yield and the highest levels of saponins and flavonoids. This study compares maceration, reflux and soxhlet extraction methods with a variety of solvents including *N-Hexanee, Etyl Acetate* and 96*% ethanol*. Compounds from plants can be obtained through an extraction method that aims to obtain the active substance content of these natural ingredients by using an appropriate solvent. Various kinds of extraction techniques have been developed ranging from the use of simple tools to the use of modern tools.

Based on the data above, researchers are interested in comparing the yield of clove leaf and flower extracts, especially the content of flavonoids and saponins that can function as biolarvicides. The purpose of this study was to analyze clove leaf and flower extracts as an alternative to *Ae. aegypti* larvicides.

MATERIALS AND METHODS

The method used in this research is to conduct analyze clove leaf and flower extracts as an alternative to *Ae. aegypti* larvicides at the Laboratory of Pharmacy/Biological Biology I UPT Lab Bhakti Kencana University. The materials used were clove leaves and flowers of the *Zanzibar* variety obtained from plantations in Bandung Regency. The research that has been carried out uses clove leaves and flowers as research samples obtained from clove plantations in the Bandung Regency area. The cloves used are of the Zanzibar variety.

The yield of an extract can be influenced by several factors, one of which is through cold and hot extraction

Table 1. Phytochemical Contents of extracts of leaves and flowers of cloves from Soxhlet extraction

Phytochemical Screening	Result	
	Leaf	Flower
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tanin	+	+
Kuinon	+	+
Steroid/ Triterpenoid	+	+

Primary Data, 2021

methods. Extraction with solvent variations consisted of cold methods including maceration, percolation and hot methods including reflux, soxhletation, infusion, decoction and digestion. The solvent used is based on the ability to dissolve the active substance in the maximum amount, so that an extract is formed (the extraction product containing various chemical components). The yield uses percent (%), which means that the higher the yield value, the more the extract value is produced.

The method used in this study was to analyze the yield of clove flower and leaf extracts through maceration, reflux and Soxhlet methods with a variety of 3 solvents, namely *N-Hexanee, Etyl Acetate* and 96% *ethanol*. Determination of the levels of flavonoid content using a spectrophotometer while the determination of saponin levels using the gravimetric method.

Process with the Reflux method, 50 grams of clove leaf or flower powder is weighed and put into a round bottom flask, then 100 mL of solvent (N-Hexane*e or ethyl acetate or ethanol*) is added and heated at 60°C for 3 hours, then filtered using a Buchner funnel. The liquid extract obtained was evaporated in a rotary evaporator to obtain a thick extract. *Soxhlet* method, 50 grams of powder or cloves are weighed and wrapped in filter paper, tie both ends with thread, then put it in a soxhlet tube (thimble), add a variety of solvents (solvent of *N-Hexanee* or *Etyl Acetate* or 96% *ethanol*) as much as 100 ml. The extraction process was carried out at 70°C until the cycle drops became clear. The liquid extract obtained was evaporated on a rotary evaporator to obtain a thick extract.

RESULT

Based on the results of the research that has been carried out, the data obtained are as follows (Table 1):

The Table 1 shows, in the Phytochemical analysis with extraction from the Soxhlet process. It was found that the clove extract in both leaves and clove flowers contained flavonoids, saponins, and tannins which were larvicidal compounds. The process of extracting cloves leaves and flowers in this experiment used maceration, reflux and soxhlet methods with various solvents, namely *N-Hexanee, Etyl Acetate* and 96% *Ethanol*. The clove leaves and flowers used are from Bandung Regency, precisely in the Cimaung area. The type of clove used is Zanzibar. The price of 1 kg of clove leaves is Rp. 2100, - and the price of 1 kg of clove flowers is Rp. 60,000.

The Table 2 shows the results that obtained at the extraction stage. It showed that the highest extract yield was found in the leaves with a reflux process with ethyl acetate solvent, which was 20.36%. The reflux process is an extraction process by heating. Factors that can affect the extraction process are the amount of solvent and extraction time. Meanwhile, in the Soxhlet method, the yield of 6.93% was obtained on flowers with N-Hexane pellets. In the maceration process, the extract content of the leaves with Ethyl Acetate solvent was 16.80%.

The three figures below will further clarify the comparison of the yield of clove leaf and clove flower extracts through maceration, reflux and sokhlet methods. The figure can be seen in Figure 1, Figure 2 and Figure 3. Each extraction method is carried out with a variety of solvents. The solvents are N-Hexane, Ethyl Acetate and Ethanol 96%.

Table 3 shows that the highest levels of flavonoids in the maceration process with 96% ethanol solvent obtained levels of 23.31% in clove leaves. The highest levels of flavonoids were found in the leaves through a reflux process with 96% Ethanol solvent levels of 29.53%. And the highest levels of flavonoids produced through the Soxhlet process with 96% ethanol solvent were found in the leaves, which was 19.83%.

The two figures below will further explain the comparison of flavonoid levels from clove leaves and clove flowers extracted in each method. The methods used are maceration, reflux and Sokhlet. With each method using a variety of solvents. The solvents include N-Hexane, Ethyl Acetate and Ethanol 96%. This figure can be seen in Figure 4 and Figure 5.

The Table 4 shows that the highest levels of saponins were found in the leaves through the *Soxhlet* process with 96% ethanol as solvent, 9.92%. Meanwhile, in the maceration method, the highest saponin content was found in leaves with a concentration of 9.6% using 96% ethanol as solvent. And in the reflux method, the highest saponin content was found in leaves with a concentration of 2.88% using *N-Hexanee* ethanol as solvent.

Table 2. Comparison of the yield of clove leaf and flower extracts with variations of 3 methods and 3 solvents

Extraction Method of Sample Solvent	Sample	Solvent	Weight of Simplicia (g)	Weight of Extract (g)	Extract Yield (%)
Maserasi	Leaf	*N-Hexane*	100 g	4.0909 g	4.09%
		Etyl Acetate		16.8046 g	16.80%
		Ethanol 96%		6.9792 g	6.98%
	Flower	*N-Hexane*	100 g	7.3975 g	7.40%
		Etyl Acetate		10.2687 g	10.27%
		Ethanol 96%		1.9259 g	1.93%
Refluks	Leaf	*N-Hexane*	100 g	4.3017 g	4.30%
		Etyl Acetate		20.36 g	20.36%
		Ethanol 96%		1.728 g	1.73%
	Flower	*N-Hexane*	100 g	4.5234 g	4.52%
		Etyl Acetate		5.3765 g	5.38%
		Ethanol 96%		9.3707 g	9.37%
Sokhlet	Leaf	*N-Hexane*	50 g	5.8745 g	5.87%
		Etyl Acetate		4.0202 g	4.02%
		Ethanol 96%		2.4785 g	2.48%
	Flower	*N-Hexane*	50 g	6.9346 g	6.93%
		Etyl Acetate		4.3401 g	4.34%
		Ethanol 96%		3.8638 g	3.86%

Primary Data, 2021

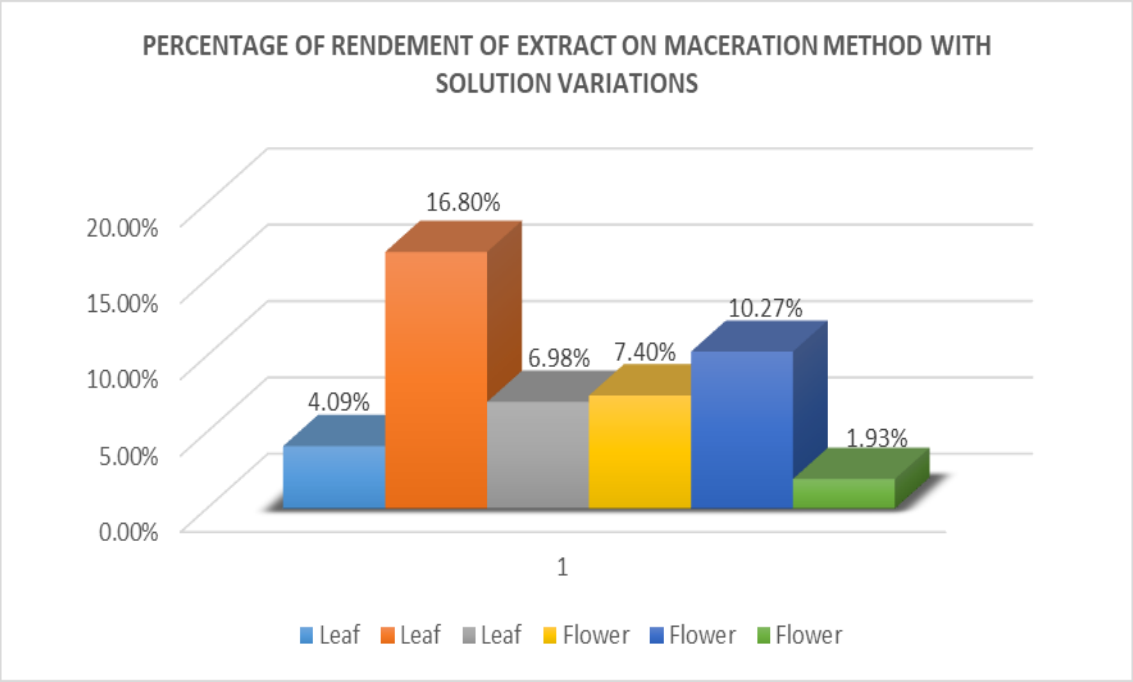

Figure 1: Percentage of rendement of extract on maceration method with solution variations

Primary Data, 2021

Figure 6 and Figure 7 below will further explain the comparison of Saponin levels from clove leaves and clove flowers extracted using various solvents. The solvents are N-Hexane, Ethyl Acetate and Ethanol 96%. Where the analyzed extract was obtained from extraction through maceration, reflux and Sokhlet methods.

DISCUSSION

The best extraction method is a method that is able to produce leaf and flower yield extracts with the highest percentage of extract yields, the highest levels of *saponins* and *flavonoids*. In the initial extraction process, the highest

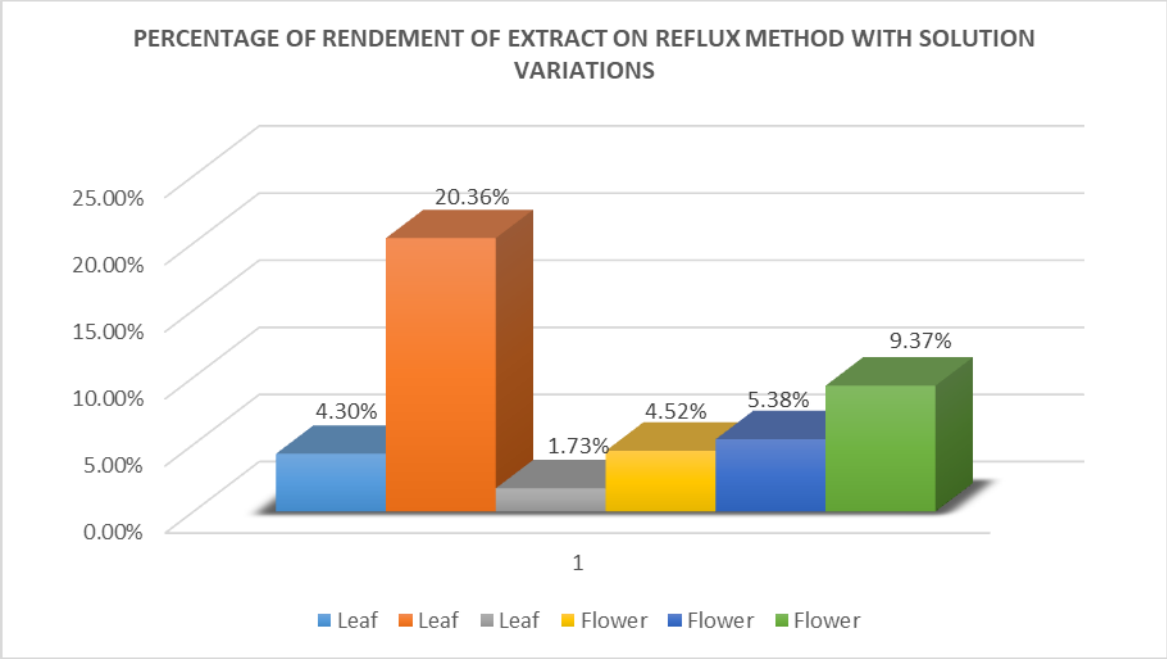

Figure 2: Percentage of rendement of extract on reflux method with solution variations

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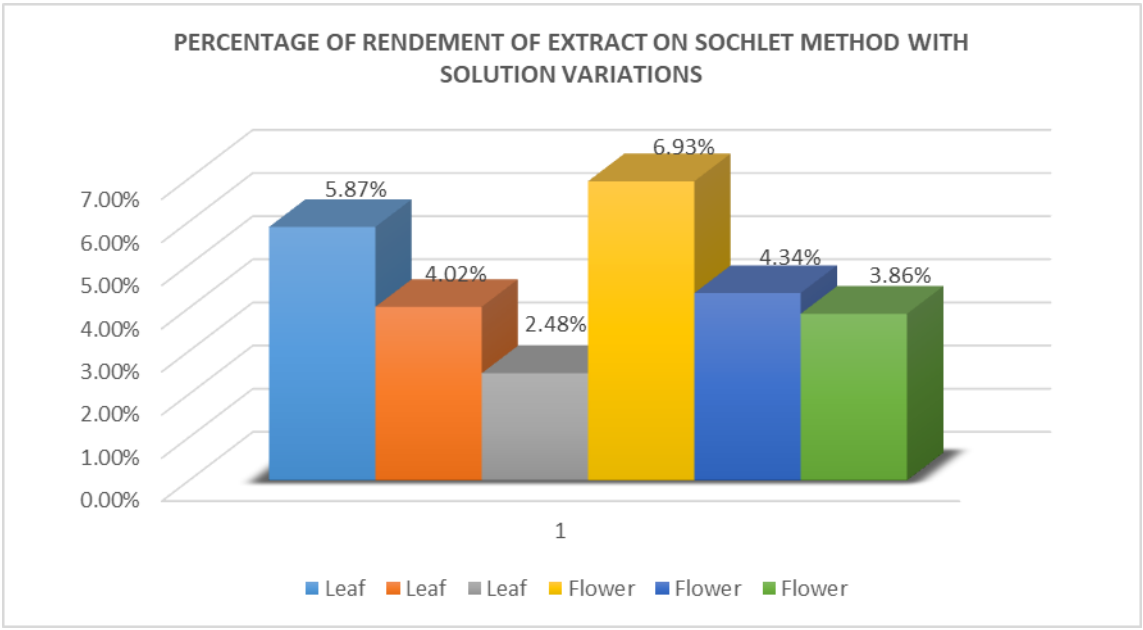

Figure 3: Percentage of rendement of extract on sochlet method with solution variations

Primary Data, 2021

extract yield was found in the leaves by refluxing with Ethyl Acetate solvent, which was 20.36%. And the highest levels of flavonoids are found in the leaves through a reflux process with 96% Ethanol solvent levels of 29.53%.

The reflux method makes it easier for the contents of the cell to come out, because with this method of hot extraction, the cell walls are easily broken. The principle of the reflux method is that the solvent used will evaporate at a high temperature, then cooled by a condenser so that the solvent in the form of vapor will condense in the condenser and fall into the reaction vessel where the solvent will remain during the reaction. Then, the solution was filtered using a

Table 3. Levels of Flavonoids in Clove Leaves and Flowers with Variations of 3 Methods and 3 Solvents

Sample	Method	Solvent	Concentration µg/ml	Absorbance	% Concentration
Flower	*Maserasi*	*N-Hexane*	5000	0.246	2.26
		Ethyl Acetat	800	0.294	3.39
		Ethanol 96%	1000	0.224	2.17
	Reflux	*N-Hexane*	1000	0.222	2.16
		Ethyl Acetat	1000	0.364	3.5
		Ethanol 96%	1000	0.391	3.63
	Soxhlet	*N-Hexane*	300	0.432	13.80
		Ethyl Acetat	300	0.422	13.48
		Ethanol 96%	300	0.521	16.6
Leaf	*Maserasi*	*N-Hexane*	300	0.322	10.33
		Ethyl Acetat	600	0.560	8.70
		Ethanol 96%	150	0.346	23.31
	Reflux	*N-Hexane*	600	0.407	6.30
		Ethyl Acetat	200	0.336	15.54
		Ethanol 96%	150	0.463	29.53
	Soxhlet	*N-Hexane*	300	0.624	19.42
		Ethyl Acetat	300	0.574	18.26
		Ethanol 96%	300	0.611	19.83

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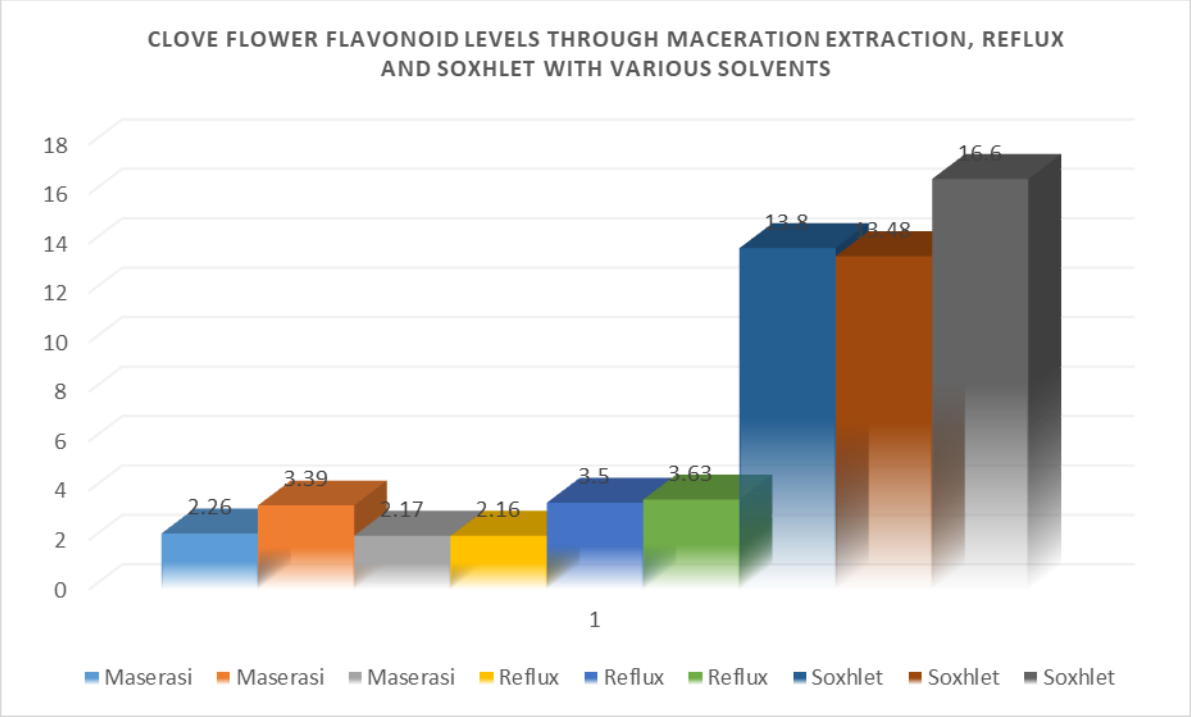

Figure 4: Clove Flower Flavonoid Levels Through Various proces and Various Solvents

Primary Data, 2021

filter cloth. The filtrate was evaporated using a rotary evaporator and then dried in an oven at 50°C for 2 days, in order to obtain a dry extract. This is done so that the solvent runs out so that the solvent does not affect the effectiveness of the sample being tested. Furthermore, the extract was dried in the oven for approximately 5 days to reduce the water content contained in the extract. The yield obtained is in the form of dry extract.

The reflux process was carried out for 3 hours at a temperature of 60°C to prevent the possible decomposition of substances that are not resistant to high temperatures. The use of time greatly affects the extraction process. The

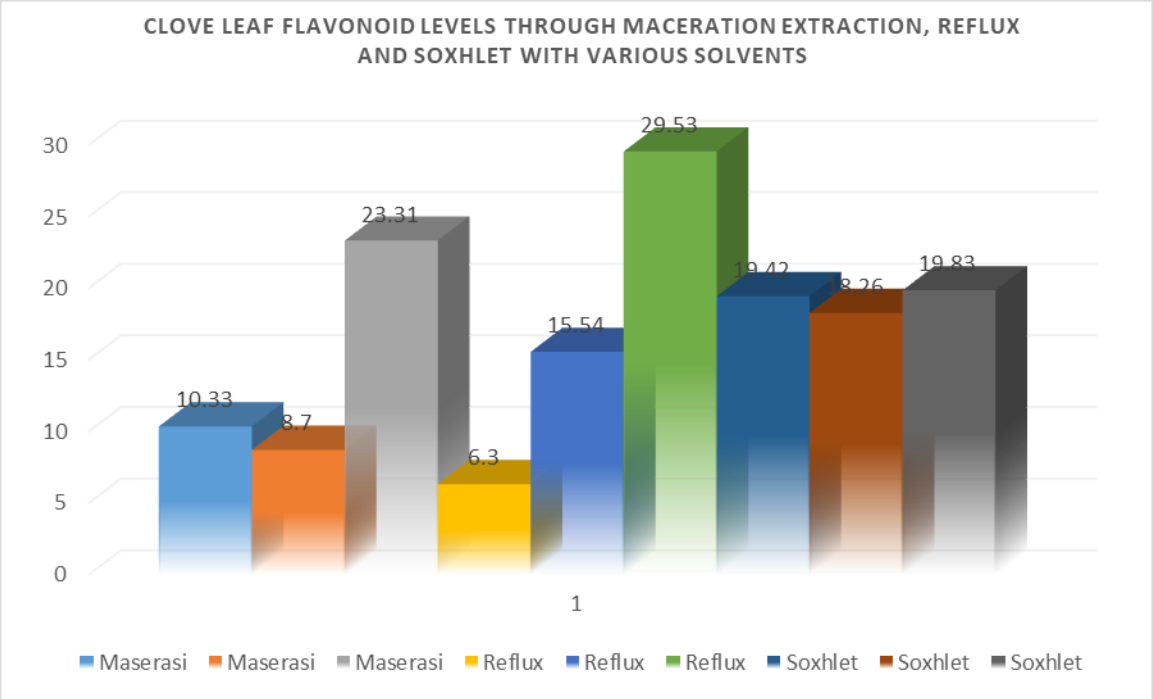

Figure 5: Clove Leaf Flavonoid Levels Through Various proces and Various Solvents

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Table 4. Levels of Saponin in Clove Leaves and Flowers with Variations of 3 Methods and 3 Solvents

Sample	Method	Solvent	X1	X2	A	% Concentration
Leaf	Maserasi	N-Hexane	1.05	1.074	0.625	3.84
		Ethyl Acetat	1.04	1.058	0.625	2.88
		Ethanol 96%	1.05	1.069	0.625	3.04
	Reflux	N-Hexane	1.06	1.071	0.625	1.76
		Ethyl Acetat	1.05	1.056	0.625	0.96
		Ethanol 96%	1.04	1.053	0.625	2.08
	Soxhlet	N-Hexane	1.06	1.076	0,625	2.56
		Ethyl Acetat	1.03	1.047	0.625	2.72
		Ethanol 96%	1.05	1.086	0.625	5.76
Flower	Maserasi	N-Hexane	1.04	1.063	0.625	3.68
		Ethyl Acetat	1.04	1.048	0.625	1.28
		Ethanol 96%	1.05	1.110	0.625	9.6
	Reflux	N-Hexane	1.06	1.078	0.625	2.88
		Ethyl Acetat	1.05	1.059	0.625	1.44
		Ethanol 96%	1.06	1.067	0.625	1.12
	Soxhlet	N-Hexane	1.03	1.043	0.625	2.08
		Ethyl Acetat	1.04	1.052	0.625	1.92
		Ethanol 96%	1.05	1.112	0.625	9.92

Primary Data, 2021

use of a short time aims to prevent damage to the compounds in the sample due to prolonged heating. In the extraction process, the extraction time is very influential on the results obtained. Mardina in Wijaya stated that the longer the extraction time, the higher the yield obtained, because the opportunity to react between the material and the solvent is getting longer so that the solvent penetration process into the material cell is getting better which causes more compounds to diffuse out of the cell (Wijaya et al., 2018).

The extract yield in the reflux method has a higher yield than the extract in the maceration and soxhlet methods,

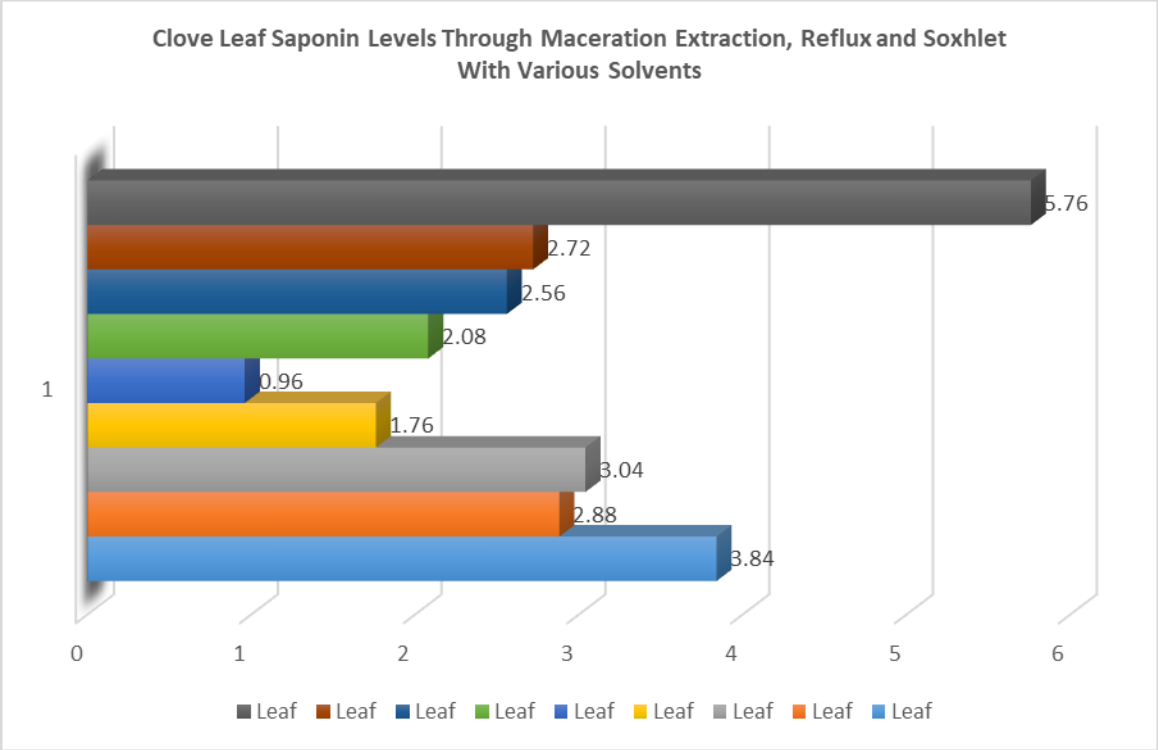

Figure 6: Clove Leaf Saponin Levels Through Various process and Various Solvents

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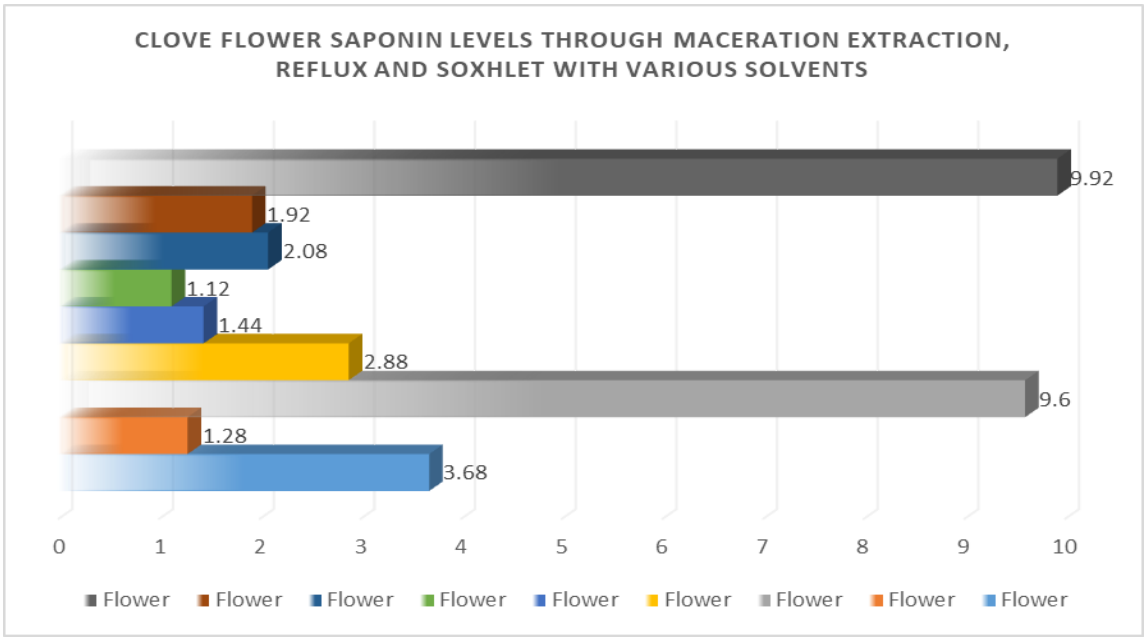

Figure 7: Clove Flower Saponin Levels Through Various process and Various Solvents

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both on leaves and flowers. Meanwhile, the highest yield of extracts from flowers using the same method was obtained from extraction with 96% ethanol solvent of 9.37%. According to Suryanto in Susanti, the extraction result will

be influenced by several factors, including the type of solvent, the concentration of the solvent, the particle size of the simplicia, and the length of the extraction time. The success of the separation depends on the difference in the solubility of the components to be separated in the solvent (Susanty, 2016).

The solvent in this case plays an important role in extracting natural materials. The choice of solvent based on its solubility and polarity facilitates the separation of natural substances in the sample. Polar compounds dissolve in polar solvents, and vice versa. Flavonoid compounds and saponins are polar, this is what makes the highest levels achieved in 96% ethanol solvent. In addition to the type of solvent, sample size also affects the amount of yield. The smaller the surface area of the sample will further expand the contact and increase the interaction with the solvent (Sineke et al., 2016).

The use of 96% ethanol in the reflux process resulted in the highest flavonoid content compared to other methods or solvents in determining flavonoid levels. Based on Ganjar's research in Sineke, it was stated that the solvent used in this study was 96% ethanol because secondary metabolites contained in clove leaves were best attracted to that concentration. In addition, ethanol is a type of solvent with good extraction capabilities for almost all chemical compounds that have small molecular weights such as secondary metabolites (Sineke et al., 2016).

The highest levels of saponins were found in the leaves through the Soxhlet process with 96% ethanol as solvent, 9.92%. Soxhlet extraction method takes longer time, this is because the extraction process is carried out continuously. Screening is done repeatedly with a relatively constant amount of solvent, causing the chemical components or compounds in the sample to be well isolated. Soxhlet extraction method is often used because the extraction process occurs perfectly so that the extract obtained is also more and with a heating process that can help speed up the extraction process.

Setyowati in Wijaya, stated that the higher the extraction temperature, the faster the molecular movement, as well as the circulation (movement) of the solvent. The presence of temperature factors and solvent circulation can increase the rate of mass transfer of compounds from leaf cells, thus the contact of the solute in the sample with the solvent is more frequent and more extract is obtained (Wijaya et al., 2018).

According to Ratna's research, PSN 3M Plus needs to always be socialized as a community-based program in an effort to prevent DHF. The PSN 3M Plus program will be more complete if the plus which is used as an alternative can be carried out by the community independently, including the use of natural materials as biolarvicides (Kurniawati, 2020).

Conclusion

The results showed that the highest yield of extract was obtained from the leaves through a reflux process with Etyl Acetate solvent, resulting in the largest extract yield of 20.36%. The highest levels of flavonoids were found in the leaves through a reflux process with 96% Ethanol solvent levels of 29.53%. The highest levels of saponins were found in the leaves through the Soxhlet process with 96% ethanol as solvent, 9.92%.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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