The Effectiveness Of Shampoo Based On Kaffir Lime Leave Extract (*Citrus Hystrix* DC) And Lime Leave (*Citrus Aurantifolia*) As Natural Protection Against Head Lice

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Abstract.

Pediculus humanus capitis is one of the problems that can interfere with human health. To overcome this, alternative treatments other than commercial shampoos are needed that can harm humans. The selection of shampoo from natural ingredients can be an alternative, namely Kaffir lime leaf shampoo (Citrus hystrix DC) and lime leaf shampoo (Citrus aurantifolia). The purpose of this study was to determine the characteristic properties of anti-head lice shampoo and determine the optimum concentration of kaffir lime leaf extract and lime leaf extract against head lice mortality. Based on the results of research for phytochemical screening show that the extract contains flavonoid compounds, saponins, alkaloids, triterpenoids, and tannins. The characteristics of shampoo preparations are that they have a blackish-green shampoo color, a distinctive aroma of orange leaves, and a thick form. In addition, a normal pH of 5-6, viscosity of 100-6000 cP, and foam height of 4-6 cm is obtained. Effectiveness tests against head lice were obtained at an optimum concentration of 5% for both kaffir lime leaf extract and lime extract. This shows the good potential of natural ingredients kaffir lime leaves and lime leaves as the basic ingredients of anti-shampoo lice.

Keywords: *Anti-lice shampoo; kaffir lime leaf (Citrus hystrix DC); lime leaf (Citrus aurantifolia) and Pediculus humanus capitis.*

I. INTRODUCTION

Pediculosis capitis or human head lice triggered by Pediculus humanus capitis is a public health concern worldwide, including in developing and developed countries [1], [2]. Human head lice spread widely as a pandemic by parasitizing many schoolchildren in different countries [3]. Pediculosis capitis was reported in 87.5% of schools, with the most cases recorded in children aged 6–9 years (68%) [4]. Using a randomized effect model, it is estimated that the prevalence of pediculosis capitis in school students reaches about 19% (confidence intervention 95% = 0.18–0.20%, I 2 = 99.89%) [5]. The most dominant factor in the incidence of pediculosis capitis is personal hygiene [6], [7]. It is shown from research that children with poor personal hygiene have an 8,713 times risk of developing pediculosis capitis compared to children with good personal hygiene [8].Head lice live off feeding on human blood [9]. They can result in significant blood loss, especially in cases of severe and persistent infestations. This loss of blood can lead to anemia, a condition characterized by a decrease in the number of red blood cells or hemoglobin in the blood. [9]–[11]. In addition, the presence of head lice can cause an itchy sensation due to sensitivity to the dirt produced by lice or as a result of their bites. Scratching can cause damage to the skin, reduce the integrity of the skin, and trigger the onset of secondary bacterial infections [4], [10].Treatment of head lice infestations generally relies on insecticidal products, but the use of these products has led to increased resistance in the head lice population [2], [3].

As a result, head lice in different parts of the world have developed resistance to insecticides, prompting research and development of various alternative treatment methods. Such alternative methods include the use of plant extracts as well as natural and synthetic oils [12], [13]. Some plant products offer the potential to produce new compounds that are effective in treating head lice infestations [14]. Medicinal plants are increasingly used in the manufacture of shampoos as an effort to cope with head lice infestation

[15]–[17]. This approach is increasingly in demand because head lice are increasingly resistant to chemicals used in treatment, as well as because of the need for safer, more effective, and environmentally friendly alternatives [14], [17], [18]. Natural products have become an option in traditional medicine. Interest in its use is increasing because the price tends to be more affordable and considered safer by the public [14], [19].Several studies have tested the effectiveness of herbal shampoos containing ingredients from plants native to Thailand such as Acorus calamus Linn., Phyllanthus emblica Linn., and Zanthoxylum limonella Alston, showing promising results both in vitro and in vivo [20], [21]. In addition, other plants such as soursop leaves (Annona muricata L.) are known to contain acetogenin compounds that are effective in killing head lice [22]. The utilization of these natural ingredients provides a potential solution to the problem of head lice resistance and also helps reduce the risk of side effects associated with the use of chemical treatments.

From the literature review, it was found that Indonesia also has a variety of natural resources that can be utilized as natural insecticides [16], [17]. Some natural ingredients that stand out in their ability to overcome head lice infections, such as lime (Citrus hystrix), red betel (Piper ornatum), crimson (Annona squamosa), kirinyuh (Chromolaena adorate), pandan wangi (Pandanus amaryllifolius), bay leaves koja (Murraya koenigii L.), duku (Lansium domesticum corr), soursop (Annona muricata L.), eucalyptus (Melaleucalucalus L.), eucalyptus (Melaleuca cajuputi), garlic, shallots, coconut (Cocos nucifera), sembung rambat (Mikania micrantha), aloe vera (Aloe vera), jarak pagar (Jatropa curcas L.) and mengkudu (Morinda citrifolia) [16]. These plants contain compounds that function as insecticides such as flavonoids, tannins, alkaloids, saponins, steroids, and essential oils that can reduce the number of lice by disrupting the digestive process as well as causing infertility and inhibiting the development of lice [23], [24]. However, not all of these natural ingredients are used as basic ingredients for shampoo to be more effective and easy to use by the community. Therefore, research was conducted by utilizing natural ingredients as the basic ingredients for making shampoo, focusing on making anti-lice shampoo. This study used kaffir lime peel leaf extract and lime leaf extract as the basic ingredients of shampoo, to evaluate the effectiveness of shampoo formula in combating head lice. The hope is to create an alternative to anti-lice shampoo that is safe, affordable, and environmentally friendly.

II. METHODS

Tools and Materials

The tools used in this study include the Thermo Fisher *Scientific IS50* Infrared Spectrophotometer (FTIR), *rotary evaporator* RE-100 Pro D-LAB, Kern ABJ analytical balance, Brookfield viscometer type RVT (VM-BF-RV-01), pH meter, *hotplate*, blender, glassware.The materials used in this study were aquades (H_2O), hydrochloric acid (HCl) p.a 2 N, iron (III) chloride ($FeCl_3$) 5%, kaffir lime leaves (*Citrus hystrix* DC), lime leaves (*Citrus aurantifolia*), ethanol (C_2H_5OH) 96%, *Hydroxypropyl methylcellulose* ($CH_3CH(OH)CH_2$), filter paper, *methyl parabens* ($C_8H_8O_3$), sodium lauryl sulfate, Dragendroff reagent, Mayer reagent, Liebermann-Burchard reagent and Wagner reagent, head lice (*Pediculusis humanus capitis*).

Procedure

Extraction of Kaffir Lime Leaves (*Citrus hystrix* DC) and Lime Leaves (*Citrus aurantifolia*)

Samples of kaffir lime leaves and lime leaves were thoroughly washed and dried at room temperature, respectively. Then, cut into small pieces and mashed with a blender. Each sample weighed 1 kg, and 96% of the ethanol solvent was added until submerged; then, maceration was carried out in a closed container for 24 hours. The filtrate is filtered with gauze, and the pulp is re-soaked in the same solvent. This process is carried out three times. The filtrate is collected and evaporated on a rotavapor at 59 ^oC until a thick extract is obtained [22].

Phytochemical Screening

Phytochemical screening serves to determine the contents of secondary metabolites in the sample. Phytochemical screening includes flavonoid test, saponin test, tannin test, alkaloid test, and terpenoid test. The flavonoid test was carried out by taking extracts from all three samples of 3 drops each, inserted in a drip plate, and adding 5% $FeCl_3$. Then, it observed its color change. The saponin test is carried out by taking

extracts of samples of 2 mL each and adding water as much as 10 mL, then shaking vigorously. If foam or foam persists within 10 minutes, it indicates saponins' presence. The tannin test takes sample extracts of 3 drops each into a drip plate. Then, two drops of 1% solution of iron (III) chloride ($FeCl_3$) are added, and discoloration is observed. If the solution changes color to blue or blackish-green, tannin compounds are present in the sample. The alkaloid test was performed by taking a sample extract of 3 drops each into a drip plate, then adding two drops of Mayer's reagent, Wagner's reagent, and Dragendroff's reagent and observing the color change. The terpenoid test was carried out by inserting sample extracts of 3 drops each into a drip plate, adding Liebermann-Burcahrd reagent, and observing color changes [25], [26].

FTIR Analysis

FTIR analysis was conducted to determine the functional groups that indicate the content of citronellal compounds in lime leaf extract and limonoid compounds in lime leaf extract. Each sample is crushed until smooth and then put into a mold so that a pellet is formed and analyzed at a 400-4000 cm-1 wavelength.

Shampoo Formulation

The shampoo is done by weighing all the ingredients used according to the formulation presented in Table 1. Then, the aqueous is heated and added to sodium lauryl sulfate until it dissolves. Then, hydroxy propyl methyl cellulose (HPMC) was crushed until thick. Furthermore, methylparaben is dissolved with ethanol in small amounts until dissolved, and then it is added to a container containing a mixture of sodium lauryl sulfate and HPMC. The mixture is stirred until homogeneous, and each lime leaf extract and lime leaf are added and stirred until homogeneous. Then, it was put into a beaker and added aqueous up to a volume of 50 mL and homogenized [22], [27].

Table 1. Anti-Flea Shampoo Formula

Material	Formulation				
	F0	**F1**	**F2**	**F3**	**F4**
Samples (kaffir lime leaves and lime leaves)	0	3	5	7	9
Sodium lauryl sulfate	4	4	4	4	4
HPMC	1	1	1	1	1
Methyl paraben	0,15	0,15	0,15	0,15	0,15
Aquades	50 mL	50 mL	50 mL	50 mL	50 mL

Information:

F0: 0% Formulation
F1: 3% formulation
F2: 5% Formulation
F3: 7% Formulation
F4: 9% Formulation

Shampoo Properties test

Shampoo properties test include organoleptic tests, homogeneity, pH stability, foam height, and viscosity tests, which identify shampoo preparations by looking at the shape, color, and aroma. The homogeneity test was carried out by shaking the shampoo preparation in such a way and then observing whether or not the shampoo preparation was homogeneous, which was carried out with three repetitions. The pH stability test was carried out by weighing the sample extract as much as 1.00 grams, dissolving it in 10 mL aquades, and then measuring with a digital pH meter, which was carried out with three repetitions. The viscosity test was performed using a Brookfield viscometer using 50 rpm in 100 mL. The foam height test is performed by inserting shampoo into a test tube as much as 0.1 grams. Then, add as many as 10 mL of aquades and shake the test tube for 20 seconds [22].

Test the Effectiveness of Anti-Fleas

Testing the effectiveness of anti-lice was conducted by preparing the size of a petri dish filter paper and then dripping it evenly with 0.5 mL of shampoo extract. After that, five head lice were put into a petri dish and then closed and observed every 5 minutes for 2 hours 3 times [22].

III. RESULTS AND DISCUSSION

Making anti-head lice shampoo from lime leaf extract and lime leaf consists of several stages: sample extraction, phytochemical screening, analysis using FTIR, shampoo properties test, and lice effectiveness test.

Phytochemical Screening

Table 2. Phytochemical Test Results

Active compounds	Reagents	Sample	Observation	Information
Flavonoids	$FeCl_3$ 5%	Lime leaves	Blackish green	Positive
		Kaffir lime leaves	Blackish brown	Positive
Saponins	Aquades	Lime leaves	There is foam	Positive
		Kaffir lime leaves	There is foam	Positive
Triterpenoids	Liebermann-Burchard	Lime leaves	Brownish Green	Positive
		Kaffir lime leaves	Green ring	Positive
Tannins	$FeCl_3$ 1%	Lime leaves	Blackish green	Positive
		Kaffir lime leaves	Blackish Blue	Positive
Alkaloids	Wagner	Lime leaves	Black	Negative
		Kaffir lime leaves	Black	Negative
	Mayer	Lime leaves	Brownish-green	Negative
		Kaffir lime leaves	Black	Negative
	Dragenroff	Lime leaves	There are deposits	Positive
		Kaffir lime leaves	There are deposits	Positive

Phytochemical screening is carried out to determine the content of secondary metabolite compounds contained in shampoo. The test results on kaffir lime leaf extract showed the presence of flavonoids, saponins, terpenoids, tannins, and alkaloids. Shampoo extract reacted with a 5% $FeCl_3$ solution is characterized by a change in color to a brownish-black color [28]. Testing saponin compounds with water found the presence of foam in the shampoo extract. The foam contained in saponins indicates that saponins are compounds referred to as natural surfactants, so their properties are similar to detergents. Saponins are polar solvents that dissolve in water solvents. In addition to having polar properties, saponins also have nonpolar properties because they have a hydrophobic group, namely sapogenin. Tannin content is characterized by a blackish-green color, triterpenoids are characterized by the presence of a brownish-green color, and the presence of alkaloid content is characterized by the formation of white or orange deposits with the addition of Dragendroff reagent [29]. The presence of this metabolite compound in shampoo can act as a poison that can inhibit growth and kill insects by damaging cell membranes and disrupting metabolism [30].

Functional Group based on FTIR Spectrophotometer

Testing using an FTIR Spectrophotometer aims to determine the functional groups contained in the sample. Based on the readings, functional groups were obtained that showed the presence of limonoids contained in lime leaf extract, including O-H *stretching* (2926.44 cm^{-1}), COO (1730.64 cm^{-1}), C=O *stretching* (1613.12 cm^{-1}), C-O *stretching* (894.26 $^{cm-1}$), C-O ether (1155.72 cm^{-1}), O-Me methoxy (1075.23 cm^{-1}), C-H *stretching* (620.22 cm^{-1}).The FTIR results of kaffir lime leaf extract include OH *stretching* (2864.76 cm^{-1}), COO (1732.66 cm^{-1}), C=O *stretching* (1627.38 cm^{-1}), C-O stretching (914.52 cm^{-1}), C-O ether (1129.02 cm^{-1}), Methoxy O-Me (1073.63 cm^{-1}) and C-H stretching (631.26 cm^{-1}). According to research [31] which states that the basic structure of a limonoid can be known if it has O-H functional groups *stretching*, COO, C=O *streching*, C-O *streching*, C-O ether, O-Me methoxy, and C-H *streching*. Limonoid has the potential as a natural insecticide because of their ability an inhibit head lice from eating their food [16].

Properties of Shampoo

Table 3. Properties of Head Lice Shampoo

Sample	Properties	Concentration (%w/v)				
		0	3	5	7	9
Lime leaves	Organoleptic Color	Blackish green	Blackish green	Blackish green	Blackish green	Blackish green

	Existed	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	Smell	Odorless	Lime leaves	Lime leaves	Lime leaves	Lime leaves
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Foam height	5.06 ± 0.08	5.6 ± 0.22	6.04 ± 0.02	6.13 ± 0.024	6.46 ± 0.51
	pH	6.91 ± 0.07	6.33 ± 0.04	6.13 ± 0.02	6.04 ± 0.02	5.86 ± 0.008
	Viscosity	100 cP	300 Cp	2200 cP	3300 cP	5900 cP
Kaffir lime leaves	Organoleptic					
	Color	Clear	Blackish green	Blackish green	Blackish green	Blackish green
	Existed	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	Smell	Odorless	Kaffir lime leaves	Kaffir lime leaves	Kaffir lime leaves	Kaffir lime leaves
	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Foam height	4 ± 0	4.8 ± 0.5	5.2 ± 0.2	5.5 ± 0.4	6.7 ± 0.3
	pH	7.1 ± 0.14	5.85 ± 0.03	5.81 ± 0.01	5.74 ± 0.015	5.70 ± 0.015
	Viscosity	100 cP	700 cP	3200 cP	4600 Cp	6000 cP

Organoleptic testing is one of the physical parameters to determine shampoo stability. The organoleptic analysis is carried out by observing changes in the shampoo's shape, smell, and color because attractive colors and odors that are not rancid are related to consumer comfort when using shampoo. The study's results for the organoleptic analysis of shampoo from both extracts with a concentration of 0 found that shampoo has a clear color, no aroma, and a thick form. Shampoos with concentrations of 3, 5, 7, and 9 change color to become more concentrated and smell distinctive. The higher the extract concentration, the more concentrated the color of the resulting shampoo. In addition, the thicker the shampoo forms, the more unique the aroma is. The higher the active substance added in the shampoo preparation, the stronger the aroma and the more concentrated the color produced.The shampoo homogeneity test determines the uniformity and homogeneous arrangement in shampoo preparations. A good shampoo preparation is the mixing or dispersing of all ingredients perfectly. Another purpose of homogeneity testing is to see whether coarse particles are in the shampoo preparation and whether the components are mixed evenly. The results showed that shampoo has good homogeneity.

They are characterized by the absence of coarse grains at each concentration of shampoo. The results obtained from shampoo are as follows: under the provisions of SNI 06-2692-1992, which states that a good shampoo has a liquid form or nothing settles. The pH test aims to determine the acidity so that when used, it does not cause head irritation because it is in direct contact with the scalp. The pH test results on each shampoo extract show a tendency that the higher the extract concentration, the more acidic the pH. The higher the extract concentration, the decrease in the ability to bind water. Research by Malonda *et al*. (2017) reported that the reduction of pH in adding extract concentration is due to the greater active substance contained, which makes the pH value more acidic. The pH test results of both samples are still by SNI standard No.06-2692-1992. The normal pH range for a good shampoo that does not cause skin irritation is 5.0-9.0.The foam height test in this study was conducted to determine the ability of shampoo to produce foam. Foam is one of the factors that influence consumers' choice of shampoo. Foam can maintain the presence of shampoo on the hair, make the hair easy to wash, and prevent hair bars from sticking, which can cause tangles [32].

Foam height test results of both samples showed that the higher the concentration of shampoo extract, the higher the foam produced and influenced by surfactants, which are active ingredients in shampoos that function as foam. In addition, the foam has properties that are easily soluble in water, so the

foam will appear when shaking. The resulting foam height meets the requirements of 1.3-22 cm.A viscosity test is performed to determine the viscosity of the shampoo. High viscosity indicates that the liquid has a high viscosity. The test results of both shampoo extracts showed that the higher the concentration of the extract, the less water in the shampoo, and the higher the specific weight so that the viscosity of the shampoo. Good shampoo viscosity is found in lime leaf extract shampoo at a concentration of 7, while in kaffir lime leaf extract at a concentration of 5. This result complies with SNI rules, which state that the viscosity of a good shampoo has a value in the range of 400-4000 cP (centipoise) and meets specifications that are easy to pour into the palm and easy to spill.The results of the characterization of anti-lice shampoos containing kaffir lime leaf extract and lime leaf extract showed good results according to the quality of Indonesian national standards (SNI 06-2692-1992). Furthermore, all shampoo formulas are tested for effectiveness in killing head lice.

Table 4. The Effectiveness of Anti-Flea Shampoo

Formulation	Extract shampoo	Time (minute)						Number of dead ticks
		5	**10**	**15**	**20**	**25**	**30**	
0% shampoo	Lime leaves	-	-	-	-	1	-	1
	Kaffir lime leaves	-	-	-	-	1	-	1
Shampoo 3%	Lime leaves	1	1	1	1	1	-	5
	Kaffir lime leaves	1	1	1	1	1	-	5
Shampoo 5%	Lime leaves	1	1	3	-	-	-	5
	Kaffir lime leaves	1	3	1	-	-	-	5
Shampoo 7%	Lime leaves	4	1	-	-	-	-	5
	Kaffir lime leaves	4	1	-	-	-	-	5
Shampoo 9%	Lime leaves	5	-	-	-	-	-	5
	Kaffir lime leaves	5	-	-	-	-	-	5
Aquades	Lime leaves	-	-	-	-	-	-	5
	Kaffir lime leaves	-	-	-	-	-	-	5
Peditox	Lime leaves	1	1	1	2	-	-	5
	Kaffir lime leaves	1	1	1	2	-	-	5

They tested the anti-lice effectiveness of shampoo preparations using filter paper placed in a petri dish. Each concentration test is carried out using five ticks. The results showed that positive control of peditox with 1% permethrin content resulted in 100% mortality in lice. However, with peditox, it takes longer to kill lice than with shampoo preparations made from natural ingredients. Negative shampoo control with aquades was obtained, and no death was observed in lice. The results were that the higher the shampoo extract, the higher the mortality rate and the faster the time used to kill head lice. In line with research conducted by [27], the greater the extract's concentration, the greater the mortality rate of human head lice. Because there is an influence of extractive substances or the content of secondary metabolic compounds that are more and more contained in shampoo preparations, the content of flavonoids can act as bioinsecticides in insects. Flavonoids enter the respiratory system of insects, namely spiracles on the body surface, causing damage to spiracles and inhibiting the respiratory system of insects. In addition, flavonoids can also cause the weakening of nerve function in insects [33]. Citronellal and limonoids can enter the body of head lice and

secrete their toxic ability *to scan* in direct contact, causing head lice to lose fluid continuously and die due to dryness. The poison will make the insect's body lose its condition continuously so that it dies of drought. Lack of fluid in insects can create death in insects. The poison will enter through contact with the skin into the insect's body with intermediary fingers on the surface containing insecticide residues. Based on the results obtained, lime leaf extract and kaffir lime leaf have the potential as a basic ingredient in making anti-lice shampoo.

IV. CONCLUSIONS

Based on organoleptic test results, The characteristics of anti-head lice shampoo from lime leaf extract (*Citrus hystrix* DC) and lime leaf (*Citrus aurantifolia*) based on organoleptic test results can meet SNI rules. Organoleptic results for aroma obtained a distinctive smell of kaffir orange, thick form, and a blackish color, have good homogeneity, pH 5-6, high foam 4-7, and have a viscosity of 100-6000 cP. The optimum concentration of anti-head lice shampoo preparations from kaffir lime leaf extract (*Citrus hystrix* DC) against head lice mortality and meets the requirements as a good shampoo to use is shampoo with a concentration of 5% in lime leaf extract and a concentration of 5% in kaffir lime leaf extract. Based on the results obtained, lime leaves and kaffir lime leaves have promising potential as Basic Ingredients for making natural anti-lice shampoos.

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