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Efficacy of Shampoo Made from Bangle Rhizome Extract (*Zingiber montanum*) Against Head Lice (*Pediculus humanus capitis*)

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Abstract

Various head lice treatments with different mechanisms of action have been developed and explored over the years, with limited reports on systematic assessments of their efficacy and safety. The head lice shampoo commercial is 1% permethrin-based. Side effects of using permethrin-based shampoos include skin irritation and high resistance. The research conducted aims to present strong evidence that the use of shampoo made from bangle rhizome extract against head lice mortality is safer to use. The study used 240 head lice taken from elementary school students (8-12 years) in a school in Bekasi. The study group was divided into 6 groups: negative control group, positive control group (using permethrin-based anti-lice shampoo), shampoo treatment group made from bangle rhizome extract with dose of 0.5%, 1%, 2% and 4%. In addition to calculating the number of head lice deaths per group, the time of death was also calculated to determine LC_{50} and LC_{90}. The results showed a highly significant difference between the number of head lice deaths in the control group and the treatment group with shampoo made from bangle rhizome extract ($p<0.01$). Likewise, there was a highly significant difference for the time of death of head lice in the control group and the treatment group using shampoo made from bangle rhizome extract. Statistical analysis showed LC_{50} is 1% and LC_{90} is 3%. The effective dose of shampoo made from bangle rhizome extract is 4%, which can kill 100% of head lice within 27.5 minutes.

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INTRODUCTION

Human head lice (*Pediculus humanus capitis*) infestation is a global public health problem that affects people from all socioeconomic backgrounds.[1,2] Although there is a lack of reliable data, estimates of the prevalence of head lice infestation in school-aged children range from 5% in Europe to 33% in Central and South America.[3] Head lice infestation affects people regardless of ethnicity and age, although it is more common in children aged 7-14 years, females and vulnerable populations living in crowded environments.[1]

Head lice cause considerable discomfort, and intensive itching that can lead to sleep deprivation and excoriation-although rare, wounds on skin infected with resistant pathogenic bacteria can lead to secondary skin infections and lymphadenopathy.[4,5] In addition, affected children and their parents often suffer from social stigma, embarrassment, and low self-esteem.[6,7] Data on the spread of head lice in Indonesia are limited. However, research conducted by Karimah et al.[8] stated that the prevalence of Pediculosis capitis out of 123 research subjects was 55.3%.[8] Another study conducted by Wahdini et al.[9] in a boarding school in Bogor found that the prevalence of *Pediculosis capitis* was 88.4%.[9] While Fauzan and Nita[10] stated that the prevalence of Pediculosis capitis was more in girls than boys (65.1%).[10]

The head lice (*Pediculus humanus capitis*) is wingless, 2 mm to 4 mm long (at maturity), six-legged, and is a blood-sucking insect that lives on the human scalp.[11] Infested children usually have fewer than 20 adult lice at any one time, which live 3 to 4 weeks if untreated.[12-14] Head lice live close to the scalp surface which provides food, warmth, shelter and moisture.[12,14] Head lice feed every 3 to 6 hours by sucking blood and injecting saliva simultaneously. After mating, adult female lice can produce 5 or 6 eggs per day for 30 days, each attached to a hair shaft near the scalp.[13,14]

There are various interventions available for the management of head lice.[1] Mainstream therapies have largely relied on insecticide-based approaches for decades. However, accumulating evidence with resistance to front-line insecticide treatments such as pyrethrins, permethrin, and malathion has led to increasing initiatives to develop newer and more effective treatments to safely treat this condition.[1] Over the past decades, alternative candidates have been introduced to the market, including ivermectin,[15-17] occlusive agents (e.g., benzyl alcohol, isopropyl myristate, and dimethicone),[4,18,19] and herbal products,[20] and essential oils.[20-22]

Although drugs with new modes of action have the potential to address the rapidly growing resistance problem, in the absence of strong comparative evidence, the relative efficacy and safety of newer agents and how they compare to insecticide treatment remains unclear. As a result, the government is dedicating relatively large resources to develop new products and devise strategies to control and prevent head lice through the utilization of medicinal plants available nearby. This means that, with all the limitations of using pediculicides that have been circulated and used, which can cause resistance in head lice, the government through the Ministry of Health and the Ministry of Education encourages scientists to further develop the potential of medicinal plants that are effective in killing head lice without causing resistance, as well as being safer to use.

Bangle (*Zingiber montanum*) is a type of medicinal plant that has begun to be widely researched as a natural

ingredient to treat head lice. This is because bangle rhizome extract contains chemical compounds that can be used as anti-head lice. Padmasari et al.[23] stated that the results of phytochemical screening on 70% ethanol extract of bangle rhizome (*Zingiber purpureum* Roxb.) are saponins, flavonoids, triterpenoids, steroids, essential oils, alkaloids, tannins and glycosides.[23] The research conducted aims to make shampoo made from bangle rhizome extract with graded doses that effectively work as pediculicides for head lice (*Pediculus humanus capitis*).

MATERIALS AND METHODS

Materials

The tools used are measuring cup, beaker, Erlenmeyer, test tube, stirring rod, digital analytical balance, filter paper, petri dish, rotary evaporator, oven, blender, shampoo container, pH meter, viscometer, hot plate, and maceration container (Pyrex USA). The materials needed are bangle rhizomes and 70% ethanol. For the manufacture of shampoo, sodium lauryl sulfate, hydroxypropyl methylcellulose (HPMC), methyl paraben (Ginhong Mixer, China), distilled water, and 1% permethrin are needed.

Methods

Sample Preparation

Bangle rhizomes were purchased from the Kopro traditional market Tanjung Duren Jakarta Barat. The collected bangle rhizomes were washed, sliced thinly, then dried by aerating. The dried bangle rhizomes were blended until bangle rhizome powder was obtained. Sample preparation and research were carried out at the Research Laboratory of the Faculty of Medicine and Health Sciences, Krida Wacana Christian University Jakarta.

Head Lice Preparation

Human head lice or *Pediculus humanus capitis* were obtained from girls aged 8-12 years in Cirewed Village, Cikupa, Tangerang, Banten who were infested with head lice with the criteria of not having received therapy except with a special comb or comb. Sampling of lice from the respondent's hair involved using a special comb, then the lice along with the hair to which they were attached were placed in a bottle whose lid has been perforated to allow oxygen to enter. This was with the aim that the body of the louse remained normal when given treatment.

Preparation of 70% Ethanol Extract of Bangle Rhizome

A total of 200 g of bangle rhizome powder was weighed, then filtered. The dregs obtained were macerated with 500 mL of 70% ethanol at room temperature for 3-4 days, then filtered and the filtrate was collected. The filtrate was concentrated with a rotary evaporator at 50^{o}C to obtain an extract that still contained a small volume of solvent. The evaporation of the extraction solvent was continued using an oven at 40^{o}C until a thick bangle extract was obtained.

Shampoo Preparation

The shampoo formula consists of HPMC (Hydroxy Propyl Methyl Cellulose), sodium lauryl sulfate, methyl paraben and distilled water. The shampoo added bangle rhizome extract with concentrations of 0.5%, 1%, 2% and 4%. The shampoo was prepared by making a mixture of HPMC (1%) which was added little by little into hot distilled water as much as 20 mL while stirring (mixture 1). Methyl paraben was dissolved using ethanol until dissolved (mixture 2). Then 50 mL of distilled water was put in a 1 L beaker and heated on a hot

plate at 60°C, then sodium lauryl sulfate was added and stirred until homogeneous. After ii was homogeneous, mixture 1 and mixture 2 were added and stirred until thickened. Then bangle rhizome extract was added according to the predetermined concentration, along with distilled water as much as 100 mL.[24] The finished shampoo was put into each bottle according to the predetermined concentration.

Research Stages

The research group was divided into six, namely the negative control group (given baby shampoo), the positive control group (given 1% permethrin-based anti-lice shampoo), the treatment group of bangle rhizome extract concentrations of 0.5%, 1%, 2% and 4%. Each group was given 10 lice, with four replications. After the head lice were collected, testing was done immediately because head lice can only survive for 24 hours after leaving the scalp.[25] Treatments were given by placing 10 lice on a Petri dish then given shampoo, and repeated up to four times for each group. Observations were made by counting the number and time of death of head lice. The indicator of a dead louse is that the louse does not move when touched gently with tweezers, or is a rigid body condition with an irregular leg position, not moving, and not responding to stimuli when touched.[26]

Data Analysis

The data obtained were then analyzed using the one-way ANOVA test and the least significant difference (LSD) test to determine the group that most effectively killed head lice in less than two hours (120 minutes). Probit test was conducted to determine the effective dose and time of death of head lice given shampoo made from bangle extract with graded doses. Data were processed using SPSS software version 27.0.

RESULTS AND DISCUSSION

The head lice used in the study were taken from the heads of elementary school children aged 8-12 years. This is because this age range is more exposed to head lice.[27]

The average percentage of head lice killed by shampoo treatments made from 1% permethrin and made from bangle rhizome extract at graded doses can be seen in Figure 1.

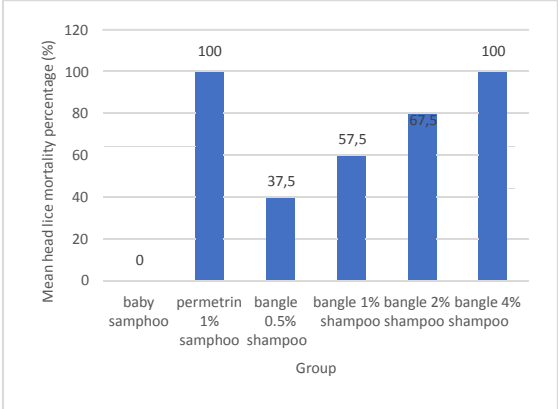

Figure 1. Mean percentage of head lice mortality in the control and treatment groups.

Based on the one-way ANOVA test in Table 1, it was found that there was a significant difference in the mean percentage of head lice mortality between the negative control group (baby shampoo), the positive control group (permethrin 1 shampoo), and the treatment group with shampoo made from bangle rhizome extract with graded doses ($p<0.01$). The test continued with the least significant difference (LSD) as shown in Table 2, the results of which stated that the negative control group was significantly different from the positive control group and the treatment group with shampoo made from bangle rhizome extract in graded doses ($p<0.01$), while the positive control group was not significantly different from the shampoo treatment group made from 4% dose of bangle

Table 1. One-way ANOVA Test of Mean Percentage of Head Lice Mortality in Control and Treatment Groups.

			Sum of Squares	df	Mean Square	F	Sig
Between Groups	(Combined)		294.708	5	58.942	103.507**	0.000
	Linear Term	Contrast	102.004	1	102.004	179.128	0.000
		Deviation	192.705	4	48.176	84.602	0.000
Within Groups			10.250	18	0.569		
Total			304.958	23			

** Significantly different (p<0.01)

rhizome extract. This means that the positive control group (permethrin 1%) and the treatment group with shampoo made from 4% dose of bangle rhizome extract showed an effective function to kill head lice (100%).

Table 2. Least significant difference test on head lice mortality rate

I Group	J Group	Mean Difference (I-J)
Baby shampoo	Permethrin 1%	-10.000*
	Bangle 0.5%	-3.750*
	Bangle 1%	-5.750*
	Bangle 2%	-6.750*
	Bangle 4%	-10.000*
Permethrin 1%	Baby shampoo	10.000*
	Bangle 0.5%	6.250*
	Bangle 1%	4.250*
	Bangle 2%	3.250*
	Bangle 4%	.000
Bangle 0.5%	Baby shampoo	3.750*
	Permethrin 1%	-6.250*
	Bangle 1%	-2.000*
	Bangle 2%	-3.000*
	Bangle 4%	-6.250*
Bangle 1%	Baby shampoo	5.750*
	Permethrin 1%	-4.250*
	Bangle 0.5%	2.000*
	Bangle 2%	-1.000*
	Bangle 4%	-4.250*
Bangle 2%	Baby shampoo	6.750*
	Permethrin 1%	-3.250*
	Bangle 0.5%	3.000*
	Bangle 1%	1.000*
	Bangle 4%	-3.250*
Bangle 4%	Baby shampoo	10.000*
	Permethrin 1%	.000
	Bangle 0.5%	6.250*
	Bangle 1%	4.250*
	Bangle 2%	3.250*

*. The mean difference is significant at the 0.05 level.

The average time of death of head lice treated with 1% permethrin-based shampoo and bangle rhizome extract at graded doses can be seen in Figure 2.

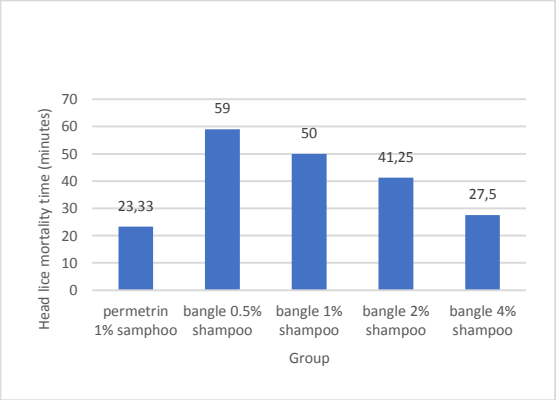

Figure 2. Mean time to death of head lice in control and treatment groups.

Based on the one-way ANOVA test in Table 3, it was found that there was a significant difference in the meantime of head lice death between the negative control group (baby shampoo), the positive control group (permethrin 1% shampoo), and the treatment group with shampoo made from bangle rhizome extract with graded doses ($p<0.01$). The test continued with the least significant difference (LSD) and, as seen in Table 4, the results stated that the negative control group was significantly different from the positive control group and the treatment group with shampoo made from bangle rhizome extract in graded doses ($p<0.01$), while the positive control group was not significantly different from the treatment group with shampoo made from 4% dose of bangle rhizome extract. This means that the positive control group (permethrin 1%) and the treatment group with shampoo made from 4% dose of bangle rhizome extract had an effective time to kill head lice (within 23 minutes and 27 minutes, respectively).

Figure 1 shows that 1% permethrin-based shampoo (positive control) was the most effective pediculicide with 100% head lice mortality at 23 minutes, followed by 4% bangle rhizome extract-based shampoo which showed 100% head lice mortality at 27 minutes. As for the shampoo made from 2% bangle rhizome extract (67.5% head lice died within 41 minutes), shampoo made from 1% rhizome extract (57.5% head lice died within 50 minutes), and shampoo made from 0.5% bangle rhizome extract (37.5% head lice died within 59 minutes). Meanwhile, the negative control group using baby shampoo showed no head lice eradication effect, with 100% of head lice surviving throughout the study.

Permethrin used as a positive control in the study is a type of synthetic pyrethroid marketed in shampoos at a concentration of 1%. It has been used to treat head lice for more than 50 years, but has recently received greater attention due to its toxic side effects and resistance.[28-30]

Resistance to the use of pyrethroid insecticides on head lice has been widely studied, including research conducted by Brownell et al.[31] which stated that there was a 40% mutation of head lice in Thailand.[31] Research by Roca Acevedo et al.[32] in Chile found 50% of head lice having mutations in the T9171 allele.[32]

The effectiveness of 1% permethrin-based shampoo as a pediculicide is due to the fact that permethrin is a synthetic neurotoxic pyrethroid that acts on the nervous system used in the treatment of pediculosis.[33,34] Its mechanism of action is to disrupt sodium channels causing depolarization, thus causing respiratory paralysis in head lice.[34] Permethrin also slows the closure of Na ion channels or canals, causing neuronal dysfunction. This causes respiratory paralysis in head lice.[30,35]

Meurer-Grimes et al.[36] gave a different statement about how permethrin works on the insect body.[36] When insects are exposed to permethrin compounds, it interacts with voltage gated sodium channels that cause sodium channels to not close so that the repolarization process occurs.[37,38] Furthermore, it will cause interference in the transmission of nerve signals and the result is that the insect has difficulty controlling its muscle movements (convulsions). Damage that interferes with muscle control can also cause paralysis and loss of ability to perform vital functions leading to insect death.[30]

The death that occurred in the head lice of the treatment group with shampoo made from bangle extract with graded doses was due to the flavonoid and saponin compounds contained therein. This is in line with the results of research

Table 3. One-way ANOVA Test of Mean Time to Death of Head Lice in Control and Treatment Groups.

			Sum of Squares	df	Mean Square	F	Sig
Between Groups	(Combined)		3589.583	4	897.396	7.561**	0.000
	Linear Term	Unweighted	31.859	1	31.859	0.268	0.000
		Weighted	229.066	1	229.066	1.930	0.000
		Deviation	3360.517	3	1120.172	9.437	
Within Groups			1780.417	15	118.694		
Total			5370.000	19			

**** Significantly different (p<0.01)**

conducted by Putri et al.[39] which states that overcoming head lice with insecticides made from medicinal plants containing flavonoid compounds can interfere with digestion. Apart from flavonoid compounds, saponin compounds can also cause head lice death. Saponins can inhibit growth, damage cell membranes, and disrupt metabolism in the body of head lice. Saponins enter the insect body by inhibiting protease enzymes, which results in decreased nutrient intake and forms protein complexes, and inhibits growth.[39]

Table 4. Least significant difference test on the meantime of head lice death

I Group	J Group	Mean Difference (I-J)
Permethrin 1%	Bangle 0.5%	-35.667*
	Bangle 1%	-26.667*
	Bangle 2%	-17.917*
	Bangle 4%	-4.167
Bangle 0.5%	Permethrin 1%	35.667*
	Bangle 1%	9.000*
	Bangle 2%	17.750*
	Bangle 4%	31.500*
Bangle 1%	Permethrin 1%	26.667*
	Bangle 0.5%	-9.000*
	Bangle 2%	8.750*
	Bangle 4%	22.500*
Bangle 2%	Permethrin 1%	17.917*
	Bangle 0.5%	-17.750*
	Bangle 1%	-8.750*
	Bangle 4%	13.750*
Bangle 4%	Permethrin 1%	4.167
	Bangle 0.5%	-31.500*
	Bangle 1%	-22.500*
	Bangle 2%	-13.750*

*. The mean difference is significant at the 0.05 level.

Based on the Probit test, the use of shampoo made from bangle rhizome extract at graded doses of up to 4%, obtained an LC_{50} value of 1%, and LC_{90} of 3%.

Table 5. Probit Test of Bangle Dosage on Head Lice Mortality

	Probability	Estimate (95% Confidence Limits)
Probit	.500	1.000
	.900	3.000
	.990	4.000

Therefore, herbal shampoo made from bangle rhizome extract with graded concentrations up to 4% is expected to be used as an alternative product to control head lice (pediculicide), because it can kill 100% of head lice.

STRENGTH AND LIMITATION

Bangle rhizomes are easily and widely found in Indonesia, including Jakarta. The limitation of this study is the difficulty of obtaining head lice in large quantities.

CONCLUSIONS

The group of head lice treated with 1% permethrin-based commercial shampoo experienced 100% mortality within 23.33 minutes, which was not significantly different from the group of head lice treated with 4% bangle rhizome extract-based shampoo (100% mortality within 27.5 minutes). The effective dose of bangle rhizome extract that can kill 50% (LC_{50}) of head lice is 1%, and that can kill 90% (LC_{90}) of head lice is 3%.

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ETCHICAL CLEARANCE

The research protocol was approved by the Health Ethics Committee of the Faculty of Medicine and Health Sciences, Krida Wacana Christian University Jakarta, NoSLKE: 1577/SLKE-IM/UKKW/FKIK/KE/VIII/2023

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CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

AUTHOR CONTRIBUTION

Conceived and designed the experiments: RPS. Peformed the experiments: RPS, AWS. Analyzed the data: MPS. Contributed reagents/materials/analysis tools: AWS. Wrote the paper: RPS, MPS.

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