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Efficacy of Thai Herbal Shampoos from *Averrhoa carambola* L., *Hibiscus sabdariffa* L. and *Passiflora edulis* Sims. for Controlling Head Lice, *Pediculus humanus capitis* (De Geer)

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Abstract Pediculosis is an infestation of the scalp caused by head lice, *Pediculus humanus capitis* (De Geer), which affects important public health in schoolchildren. Nowadays, head lice resistant to chemical pediculicide are increasing, thus alternative products from herbal shampoos or herbal cream are needed for head lice control. This study aimed to determine the efficacies of Thai herbal shampoos made from fruits of *Averrhoa carambola* L. (*A. carambola*), *Hibiscus sabdariffa* L. (*H. sabdariffa*) and *Passiflora edulis* Sims. (*P. edulis*) on mortality of head lice, at 1, 5 and 10 ml/plate by filter paper contact bioassay *in vitro* test and *in vivo* test, several infested children were treated with each shampoo and make a comparison between them and a chemical shampoo, carbaryl, by *in vitro* and *in vivo* tests. Every treatment was repeated three times. The *in vitro* results showed that the 5 and 10 ml/plate doses of *P. edulis* and *H. sabdariffa* shampoos exhibited the highest efficacy as pediculicide against nymphs with an LC_{50} of 0.85 ml/plate and LT_{50} between <0.1 to 0.20, and <0.1 to 0.47 min, respectively. LC_{50} of *P. edulis* and *H. sabdariffa* shampoos against adults were 0.85 and 1.09 ml/plate, respectively, and LT_{50} was between <0.1 to 0.83 min. From the *in vivo* test, *P. edulis* shampoo was found to be the most effective in controlling head lice with 100% cure rate after the 2nd treatment. It was more effective than carbaryl.In conclusion, *P. edulis* shampoo is an effective alternative as pediculicide for head lice treatment because it is safe: no side effects on the scalp of the subjects were observed.

Keywords: Herbal shampoo, Head lice, Pediculicide, *Pediculus humanus capitis*

Introduction

In Thailand, head lice infestation caused by *Pediculus humanus capitis* De Geer (Phthiraptera: Pediculidae) is a serious problem. Head lice is a blood-sucking insect. It feeds several times a day (every 2-3 hours) on the scalp and

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neck area and can lead to pruritus, skin irritation, superinfection and secondary bacterial infection of *Borrelia recurrentis*, *Bartonella quintana* and *Acinetobacter* spp. (Di Campli *et al*., 2012; Eroglu *et al*., 2016; Sunantaraporn *et al*., 2015). The epidemiology of Pediculosis capitis (head lice infestation) among kindergarteners and primary schoolchildren in urban and rural areas has been well documented. The most affected children were in the ages of 3-12 years old because children in this age group were likely to be in contact with other children as well as frequently used shared combs, brushes, hats and other types of headgears (Ruankham *et al*., 2016; Semmler *et al*., 2010). School girls were more frequently infested than school boys. Head louse transmission may be from direct head to head contact with an infected person or indirect via headband, hat, cap or jacket (Di Campli *et al*., 2012; Gallardo *et al*., 2012).

At present, eradication of worldwide head louse population is principally through the use of chemical pediculicides such as malathion, parathion, lindane (organophosphorus), carbaryl (carbamate), phenothrin, permethrin (pyrethroid) and Ivermectin (Gutiérrez *et al*., 2016; Ullio-Gamboa *et al*., 2017; Yang *et al*., 2009). Unfortunately, these chemical pediculicides are not effective today because their continuous and repeated uses have often resulted in development of head lice resistant (Burgess, 2009; Ullio-Gamboa *et al*., 2017). The increased level of head lice resistant to the most commonly used chemical pediculicides has resulted in multiple treatments and excessive dosing, hence caused serious human health problems (Al-Quraishy *et al*., 2015; Bagavan *et al*., 2011). Moreover, several pediculicide products need very long application time up to 8 h and are very toxic to children (Al-Quraishy *et al*., 2015; Semmler *et al*., 2017). It is also important to recognize that although all of the chemical pediculicides can kill lice, they do not reliably destroy eggs (Bragg and Simon, 2018; Semmler *et al*., 2017).

Carbaryl shampoo is a synthetic carbamate insecticide. It is one of several insecticides for head lice control in Thailand and worldwide. Carbaryl is highly toxic due to its harmful modes of action in human, head lice and insect pests, aquatic invertebrates, and mammals, which in most cases is inhibition of acetylcholinesterase enzyme in neurons.Carbaryl is a potential human carcinogen. There is some evidence that it causes cancer in human (especially in children) and domestic animals. Furthermore, there has been a concern in some regions that head lice may have developed resistance to carbaryl such as in the UK (Rassami and Soonwera, 2014; Wilsont and Foos, 2006; Wikipedia, 2018). In the same vein, malathion and permethrin do not only disrupt the immune system but are also neurotoxic (Gallardo *et al*., 2012; Soonwera, 2014; Yones *et al*., 2016). For safety reason, natural insecticides are better alternatives for controlling head lice because they are generally biodegradable, pest

specific, and nonallergic to human with low mammalian toxicity and environmental impact (Soonwera, 2014).

Many essential oils, plant extracts, and herb-based products have been suggested as alternatives for head lice control because they constitute a rich source of bioactive compounds (Bagavan *et al*., 2011; Soonwera, 2014; Toloza *et al*., 2010). In particular, herb-based compounds and plant extracts such as *Phyllanthus emblica*, *Zanthoxylum limonella*, *Syzygium aromaticum* (clove), *Lawsonia inermis*, *Vitex agnus castus*, *Solanum trilobatum*, *Melaleuca alternifolia*, *Origanum majorana* and *Kunzea ambigua* exert repellent, ovicidal and adulticidal effects on head lice.Also, essential oils from herbs such as *Cinnamomum aromaticum*, *Cananga odorata*, *Eugenia aromatic*, *Schinus areira* (Anacardiaceae), *Thymus vulgaris* (Lamiaceae), *Aloysia polystachya*, *Aloysia citriodra* (Verbenacea), *Sesamum indicum*, *Eucalyptus globulus* and *Mentha spicata* have already been widely used as local medicine in several countries (especially Southeast Asia) for controlling many insect pests such as head lice (Di Campli *et al*., 2012; Yang *et al*., 2009; Bagavan *et al*., 2011; Semmler *et al*., 2017; Toloza *et al*., 2010; Marimuthu *et al*., 2012; Rajakumar *et al*., 2014; Williams *et al*., 2016).

In this study, *Averrhoa carambola* L. (*A. carambola*), *Hibiscus sabdariffa* L. (*H. sabdariffa*) and *Passiflora edulis* Sims. (*P. edulis*) were investigated as agents for head louse control. They belong to the family Averrhoaceae, Malvaceae and Passifloracaea, respectively, and they can be cultivated throughout any tropical and subtropical areas including Thailand. Fruits of *A. carambola* have long been used in local Thai medicine for treatments of gonorrhea and diarrhea and as diuretic, oxytocic, and antipyretic.Fruits of *P. edulis* has been used as expectorant, antioxidant and anti-inflammatory, while Flowers of *H. sabdariffa* have been used in traditional Thai medicine for diabetes, hypertension, retinopathy, respiratory diseases, diuresis and reduction of cholesterol in the blood (Table 1) (Da-Costa-Rocha *et al*., 2014; Faculty of Pharmaceutical Sciences, 2018; Lim, 2012a,b; Sinthusart, 2015). This study was designed to evaluate the pediculicidal activity against head lice (*P. humanus capitis*) of Thai herbal shampoos made from *A. carambola*, *H. sabdariffa* and *P. edulis*.

Materials and methods

Plant materials

Mature fruits of *A. carambola* and *P. edulis* were picked during the summer season of 2016 (February to April 2016) from orchards in

Nakhonratchasima and Rayong provinces, Thailand, while Flower buds of *H. sabdariffa* were purchased from Chao-Krom-Poe Dispensary, Samphanthawong district, Bangkok, Thailand (Figure 1). All plant species were identified and authenticated by a plant taxonomist at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang district, Bangkok, Thailand.

Herbal and chemical shampoos

A. carambola, *P. edulis*, and *H. sabdariffa* extracts were made into shampoos by the staff of the medicinal plant laboratory, Faculty of Agricultural Technology, KMITL. These herbal shampoos were stored at 27±0.5℃ and 72±1% relative humidity (RH) as shown in Table 1. The carbaryl 0.6% (*w/v*) (Hafif shampoo®) chemical insecticidal shampoo was manufactured by IDS Manufacturing Co. Ltd., Thailand and used as a reference pediculicide for comparative toxicity tests of the herbal shampoos.

Table 1. List of herbal shampoos tested and their formulations in this study

Scientific name Common name Family	Formulation	Perported therapeutic properties	Chemical constituents
Averrhoa carambola L. Carambola Averrhoaceae	10% (v/v) crude aqueous fruits extract of *A. carambola*	Antioxidant, anti-inflammatory, analgesic, anticonvulsant, antipyretic, and anti-hyperglycemic	Pantothenic acid, lutein, α and β carotenes, vitamin C, vitamin B1 and 2 and zeaxanthin
Hibiscus sabdariffa L. Roselle Malvaceae	10% (v/v) crude aqueous fruits extract of *H. sabdariffa*	Nephroprotective activity, hepatoprotective activity, antipyretic, anti-hyperglycemic, and anticonvulsant	Delphinidin-3-O-glucoside, delphinidin-3-O-sambubioside, and cyanidin-3-O-sambubioside
Passiflora edulis Sims. Passion fruit Passifloracaea	10% (v/v) crude aqueous fruits extract of *P. edulis*	Antioxidant activity, anti-inflammatory and wound heling activities, anticancer activity and antiviral activity	Linalool, l-octanol, 1-hexanol, α-terpineol, geraniol, ethyl hexanoate

Figure 1. Three species of Thai native plants investigated in this study: fruits of *Averrhoa carambola* L. (A), *Passiflora edulis* Sims. (B) and flowers of *Hibiscus sabdariffa* L. (C)

Collection of P. humanus capitis

The protocol for collection of all stages of head lice from human beings was approved by the Institute for Development of Human Research Protections (IHRP) Ethic committee, Bangkok, Thailand (permit number 76-2558). Adults and nymphs of head lice (*P. humanus capitis*) were collected from a population of schoolgirls at the ages between 7-12 years old during the period of September 2016 to January 2017 with the approval of the directors of several primary schools in Ladkrabang and Meenburi districts, Bangkok, Thailand, and the consents of the teachers, guardians, and schoolgirls themselves. The schoolgirls had not been treated with any chemical insecticide for at least one month before the collection and they had been using only a louse comb for getting rid of head lice (Rajakumar *et al*., 2014) before treatments with our tested shampoos. Head lice were obtained and pooled by having the children comb some sections of their scalp with a louse comb and the author's team removing the lice from the comb into a clean insect box (16.5x24x5 cm) with a filter paper (Whatman® No.1; 16x24x5 cm) at the bottom. After the collection, the head lice were transported to our Laboratory at the Faculty of Agricultural Technology, KMITL, within 20 min and morphologically identified under a stereo microscope (Nikon®, SMZ-445). The identification of active adult head lice was made by an insect taxonomist at the Faculty of Agricultural Technology, KMITL. They were further used in the *in-vitro* test.

In-vitro mortality test of P. humanus capitis

Within 20 minutes of collection of head lice (nymphs and adults), *in-vitro* mortality tests were started. A contact bioassay done with Whatman®

No.1 filter paper (Rassami and Soonwera, 2013) was used to assess the pediculicide activity of the herbal shampoos (from *A. carambola*, *P. edulis* and *H. sabdariffa*), and the mortality rates that they caused against the head lice were compared to that caused by the positive control, carbaryl shampoo. The lice were exposed to three different doses of each herbal shampoo (1, 5 and 10 ml/plate (of an area of 63.58 cm^2)). The shampoos were dropped on a filter paper (Whatman® No.1, 4.80 cm in diameter). After 30-sec drying, each filter paper was placed at the bottom of a Petri dish (5.0 cm in diameter). Ten head lice (nymphs or adults) were carefully placed on each filter paper under a stereo microscope (Nikon®, SMZ-445). The head lice were then incubated at 25.5±1.1 ℃ and 71±2.5% relative humidity (RH). The mortality of the head lice on the filter papers were recorded under the stereo microscope at 10 and 60 min after they were placed on the treated filter papers. All treatments were repeated three times. The mortality criterion for the head lice were no movement of external and internal organs of head lice such as no movement of antennae, mouth, legs, thorax, abdomen, or digestive system with or without stimulation by a soft paintbrush No. 0 (Soonwera, 2014).

In-vivo mortality test of P. humanus capitis

Infested primary schoolgirls at the ages of 7 to 12 years old were selected from 5 schools in Ladkrabang district, Bangkok, Thailand. Before the selection, schoolgirls, teachers and parents were informed about the epidemiology, biology, life cycle and control of head lice. The *in vivo* test was approved by the KMITL Ethics committee, Ladkrabang, Thailand with a registered number of 2560-01-04-003 and by the Institute for Development of Human Research Protections (IHRP) Ethic committee, Bangkok, Thailand (permit number 76-2558). The schoolgirls were screened for pediculosis capitis by having them comb their scalp and neck areas with a louse comb (Gutiérrez *et al*., 2016; Rajakumar *et al*., 2014). If at least 5 head lice were found on the comb, the girl was included in the test. In all, 120 infested schoolgirls were selected to take part in the test and were randomly separated in to 4 groups (10 schoolgirls per group). All treatments were repeated three times. The treatment and control groups were the following: Group 1 was treated with *A. calamus* shampoo; Group 2 was treated with *H. sabdariffa* shampoo; Group 3 was treated with *P. edulis* shampoo; and Group 4 was treated with carbaryl shampoo (Hafif shampoo®). All infested children rubbed 20-30 ml of their respective treatment or control shampoo thoroughly on their wet scalp for 10 min and then rinsed it off with clean water. The non-pediculosis or cure rate

was checked 1 day after the treatment. Actually, the treatment was done on 3 consecutive days and the cure rate was checked after each of these 3 days.

Statistical analysis

In the statistical analysis, LT_{50}, LC_{50}, and regression equation of efficacy at 3 concentrations were determined at 95% confidence limit. The upper confidence limit and lower confidence limit were derived by probit analysis. The mean mortality rate and cure rate were used to compare the efficacies of the herbal shampoos. Significance differences were found by one-way analysis of variance (ANOVA) and Duncan's multiple comparisons with a computer program called SPSS version 16.0 (Statistical Package for Social Sciences). Differences with $P<0.05$ were defined as statistically significant. The criterion for effective pediculicidal activity of treatments in the *in vitro* test was that the median lethal time (LT_{50}) value <1.0 min (Gallardo *et al*., 2012).

Results

Pediculicidal activities found from in vitro test

The results from the filter paper contact bioassay: mortality rate, LT_{50}, and LC_{50} values of treatments at three concentrations (1, 5 and 10 ml/plate) against nymphs of head lice are shown in Table 2. At 1 ml/plate, *P. edulis* and *H. sabdariffa* shampoos were the most effective with LT_{50} values of 0.42 and 0.85 min, respectively, and 100% mortality at 60 min. At this concentration, *A. calamus* shampoo exhibited an LT_{50} value of 27.08 min and 73% mortality. At 5 and 10 ml/plate, *P. edulis* and *H. sabdariffa* shampoos exhibited LT_{50} values that ranged from less than 0.1 to 0.20, and less than 0.1 to 0.47 min, respectively, and 100% mortality at 10 min. The *A. calamus* shampoo gave an LT_{50} value ranging from 6.97 to 26.27 min and 73.32 to 93.33% mortality. These pediculicidal results showed more effectiveness than that produced by the positive control, carbaryl shampoo (80.01 to 88.01% mortality and LT_{50} values ranging from 0.28 to 0.48 min) while head lice incubated in the negative control (clean water) were found to survive to the end of the testing period. In addition, the most effective LC_{50} value at 60 min after exposure to *P. edulis* and *H. sabdariffa* shampoos was 0.85 ml/plate, followed by those of carbaryl shampoo and *A. calamus* with LC_{50} values of 1.18 and 3.45 ml/plate, respectively.

The mortality rate, LT_{50}, LC_{50} values at three concentrations of each shampoo against adults of head lice are presented in Table 3. At 1 ml/plate, *P. edulis* and *H. sabdariffa* shampoos showed effective pediculicidal activity with

LT_{50} values of 0.41 and 0.50 min, respectively, and a complete mortality rate (100%) of head lice adults at 60 min. The *A. calamus* shampoo gave an LT_{50} value of 31.14 min and 60% mortality. At 5 and 10 ml/plate, *P. edulis* and *H. sabdariffa* shampoos exhibited LT_{50} values ranging from less than 0.1 to 0.83 min and 100% mortality at 60 min. The *A. calamus* shampoo showed LT_{50} values ranging from 2.02 to 14.21 min and 80.01 to 93.31% mortality. All concentrations of carbaryl shampoo showed mortality rates ranging from 76.02 to 88.01% and LT_{50} values ranging from 0.42 to 6.30 min. The *P. edulis* shampoo was the most effective pediculicide with the lowest LC_{50} value of 0.85 ml/plate, followed by *H. sabdariffa*, carbaryl shampoo and *A. calamus* with LC_{50} values of 1.09, 2.57 and 4.18 ml/plate, respectively. The results showed significant differences (P<0.05) between every pair of treatments and the potent pediculicidal activity (LT_{50} values <1 min) of *P. edulis* shampoo.

Pediculicidal activities found from in vivo test

The results of in vivo tests are presented in Figure 2. For the 1st treatment of the shampoos, every herbal shampoo was found to be more effective for head lice control than carbaryl shampoo. The most effective pediculicide was *P. edulis* shampoo showing a cure rate of 98.0%, followed by *H. sabdariffa*, *A. carambola* and carbaryl shampoos with cure rates of 76.0, 86.0 and 84.0%, respectively. For the 2nd treatment, 1 day after the first treatment of herbal shampoos and carbaryl shampoo, all schoolgirls in the treatment groups who still had had head lice were cured. The *P.edulis* shampoo showed the most effective pediculicide activity with 100% cure rate, followed by *A. carambola* and *H. sabdariffa* shampoos with cure rates of 92.0% and 88.0%, respectively, while carbaryl shampoo showed 98.0% cure rate. For the 3rd treatment (1 day after the 2nd treatment), the *P. edulis* shampoo was still the most effective pediculicide and were more effective with 100% cure rate for head lice control than carbaryl shampoo (98% cure rate).

Table 2. Mortality of *P. humanus capitis* nymphs caused by three herbal shampoos, carbaryl shampoo, and clean water at 1, 5 and 10 ml/plate concentrations.

Treatment	Conc. (ml/plate)	% Mortality ±SD		LT_{50} (min)	95% Confidence limit (%)		LC_{50} values (ml/plate)	Regression equation	R^2
		10 min	60 min		LCL	UCL			
A. carambola shampoo	1	40.01 ±11.51e	73.32 ±11.52bc	27.08	18.62	40.31	3.45	*Y*= 12.735x+26.66	0.6631*
	5	40.03 ±20.02e	80.01 ±20.02bc	26.27	18.94	37.08			
	10	60.02 ±20.02de	93.33 ±11.52ab	6.97	0.05	13.81			
H. sabdariffa shampoo	1	96.03 ±8.91b	100a	0.85	0.81	2.03	0.85	NA	
	5	100a	100a	0.47	0.30	0.62			
	10	100a	100a	<0.1	0.29	0.55			
P. edulis shampoo	1	96.03 ±8.91b	100a	0.42	0.23	0.59	0.85	NA	
	5	100a	100a	0.20	0.07	1.35			
	10	100a	100a	<0.1	0.29	0.55			
Carbaryl shampoo (positive control)	1	80.01 ±14.10cd	84.02 ±8.91b	0.48	-	-	1.18	*Y*= 11.429x+33.6	0.4991*
	5	88.01 ±17.92bc	88.01 ±17.92b	0.28	2.48	0.37			
	10	88.01 ±17.92bc	88.01 ±17.92b	0.28	2.48	0.37			
Clean water (negative control)	1	0	0	0	0	0	NA	NA	
	5	0	0	0	0	0	NA		
	10	0	0	0	0	0	NA		

[a] Means in each row followed by different letters are significantly different ($P<0.05$, by one-way ANOVA and Duncan's multiple range test)
LT_{50} is 50% lethal time, *UCL* is upper confidence limit, *LCL* is lower confidence limit, *NA* is not available, R^2 is regression coefficient
*Significant at $P<0.05$

Table 3. Mortality of *P. humanus capitis* adults caused by three herbal shampoos, carbaryl shampoo, and clan water at 1, 5 and 10 ml/plate concentrations.

Treatment	Conc. (ml/plate)	% Mortality ±SD		LT_{50} (min)	95% Confidence limit (%)		LC_{50} values (ml/plate)	Regression equation	R^2
		10 min	60 min		LCL	UCL			
A. carambola shampoo	1	46.73 ±11.52ef	60.01 ±20.02c	31.14	14.86	101.62	4.18	*Y*=13.461x+21.34	0.7667*
	5	60.01 ±20.02cd	80.01 ±20.02bc	14.21	4.11	23.82			
	10	86.73 ±11.51ab	93.31 ±11.52ab	2.02	0.04	4.34			
H. sabdariffa shampoo	1	96.02 ±8.91b	100a	0.50	0.30	1.03	1.09	NA	
	5	96.02 ±8.91b	100a	0.83	0.73	11.7			
	10	100a	100a	<0.1	0.05	0.29			
P. edulis shampoo	1	100a	100a	0.41	0.35	0.64	0.85	NA	
	5	100 a	100a	0.20	0.15	1.59			
	10	100a	100a	<0.1	0.05	0.29			
Carbaryl shampoo (positive control)	1	76.02 ±26.10bc	88.01 ±26.80b	6.30	22.64	18.38	2.57	*Y*=11.217x+35.2	0.4667*
	5	80.01 ±28.32ab	88.01 ±26.80b	0.48	-	-			
	10	88.01 ±26.80ab	88.01 ±26.80b	0.42	0.22	0.59			
Clean water (negative control)	1	0	0	0	0	0	NA	NA	
	5	0	0	0	0	0			
	10	0	0	0	0	0			

[a] Means in each row followed by different letters are significantly different ($P<0.05$, by one-way ANOVA and Duncan's multiple range test)
LT_{50} is 50% lethal time, *UCL* is upper confidence limit, *LCL* is lower confidence limit, *NA* is not available, R^2 is regression coefficient
*Significant at $P<0.05$

Table 4. Side effects among schoolgirls after the 1st, 2nd and 3rd treatments.

Treatment	After treatment	Negative side effects		
		Red spot	Erythema	Irritation
A. carambola shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
H. sabdariffa shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
P. edulis shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
Carbaryl shampoo (positive control)	1st	yes (6.6%)	yes (6.6%)	yes (6.6%)
	2nd	yes (6.6%)	yes (6.6%)	no
	3rd	no	yes (6.6%)	no

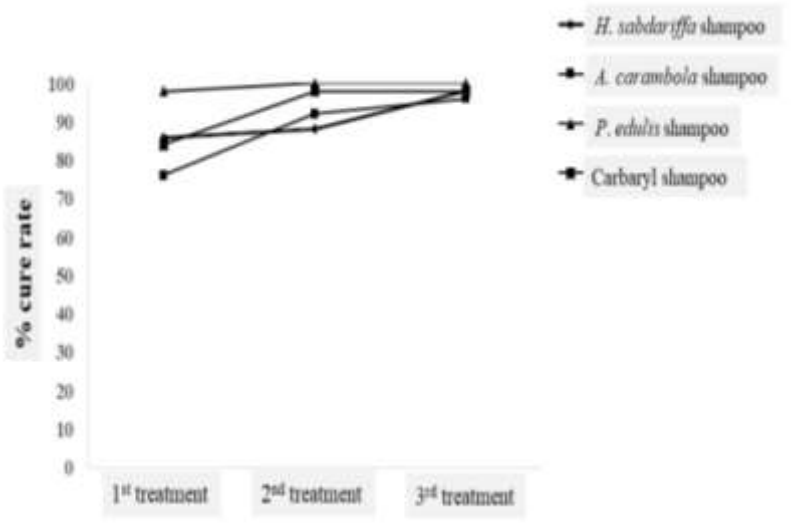

Figure 2. Percent cure rate of pediculosis capitis among schoolgirls after 1st, 2nd and 3rd treatments with tested shampoos.

Discussion

Natural herbal shampoos have been recommended as new alternative shampoos for human pediculosis capitis because they had low human toxicity and low persistence in the environment, hence a lot of efforts had been dedicated to developing them. This study has revealed that 10 ml/plate of *P. edulis* shampoo showed high pediculicidal activity against head lice (LT_{50} values < 1.0 min; *in vitro*) and was the most effective as pediculicide (100% cure rate; *in vivo*). It was more effective than carbaryl shampoo against head lice, *P. humanus capitis*.

In our investigation, *P. edulis* caused the most mortality and exhibited the highest cure rate against head lice, *P. humanus capitis*. It was found that the fruit juice with pH range of 3-4 acted as a highly effective pediculicide against head lice. This observation is supported by a work of Rassami and Soonwera (2013) that reported that 0.25 ml/cm^2 of *Averrhoa bilimbi* L. (bilimbing) and *Tamarindus indica* L. (tamarind) fruit extract effected 100% mortality at 5 min to head lice. Also, 10% crude extract of *Citrus aurantifolia* (lime) and *C. hystrix* (leech lime) also showed highly effective pediculicide activity against head lice (Watcharawit and Soonwera, 2013). The extract from *P. edulis* contained a main compound identified as linalool, which accounted for about 15.3% of all extracted substances (Table 1). The other components include l-octanol (11.5%), 1-hexanol (9.03%) and α-terpineol (Lim, 2012a). Linalool has been found to be a potent insecticide. Some chemical compounds from this extract may interfere with the central nervous system and block the respiratory system of insects (Beier *et al*., 2014; Di Campli *et al*., 2012). Yang *et al.* (2009) found that linalool was highly toxic against female head lice by a fumigation test; the LT_{50} value was 15.4 min. This compound also reduced oviposition potential of head lice, exhibiting 100% mortality 1 day after treatment by a filter-paper test. Yang *et al*. (2005) reported that a paper contact test showed that the insecticidal activity of linalool in the essential oil of *Cinnamomum zeylanicum* had a significant toxic effect against female head lice. Priestley *et al*. (2006), Candy *et al*. (2018) and Gallardo *et al*. (2009) also reported that linalool showed high pediculicidal activity against adult and eggs of *P. h.capitis* (head lice) and *P. humanus humanus* adult (body louse). Tabari *et al*. (2017) found that linalool was the most toxic compound of *Pelargonium roseum* essential oil against third-instar larvae and egg rafts of *Culex pipiens* with LC_{50} values of 14.87 and 1.27 µg/mL, respectively. Furthermore, linalool has a well-documented history of being an insect repellent.It is an effective pesticide for controlling housefly (*Musca domestica* L.) and mite (*Tyrophagus*

putrescentiae) (Beier *et al*., 2014). Müller *et al*. (2009) found that linalool diffusers repelled female mosquitoes by 93% (indoor) and 58% (outdoor).

Thus, the results obtained from the present study presented a promising scenario for using some combinations of *P*. *edulis* shampoo as an effective alternative for pediculicide for head lice treatment. Since it is an herbal shampoo, it does not have any negative side effects such as skin irritation, red spot of the scalp and neck or erythema. These were not observed after the treatments. While, 6.6% (2 of 30) of the children showed the skin irritation, red spot of the scalp and erythema after treated with carbaryl shampoo for 7 min (Table 4). The applied concentration should not be less than 10 ml/plate (20-30 ml per head). Hence, *P*. *edulis* shampoo was proven effective for head lice control.

Compliance with ethical standards

Prior to gaining consent from the participants, permission to carry out the study was requested and obtained from the Institute for the Development of Human Research Protections (IHRP) Ethics Committee, Bangkok, Thailand (permit number 76-2558).

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