International Journal of Agricultural Technology 2018 Vol. 14(4): 597-612
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Efficacy of New Herbal Shampoos from *Garcinia dulcis* Kurz, *Citrus aurantium* L. and *Eucalyptus globulus* Labill as Pediculicides for Head Lice (*Pediculus humans capitis*) Control

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Sittichok, S. and Soonwera, M. (2018). Efficacy of new herbal shampoos from *Garcinia dulcis* Kurz, *Citrus aurantium* L. and *Eucalyptus globulus* Labill as pediculicides for head lice (*Pediculus humans capitis*) control. International Journal of Agricultural Technology 14(4):597-612.

Abstract Currently, synthetic chemical pediculicides have lost their efficacy due to worldwide increased resistance of head lice to them. Therefore, safe, natural product alternatives are in dire need. This study investigated the efficacy of an herbal shampoo made from *Garcinia dulcis* (Roxb.) added with either *Citrus aurantium* EO or *Eucalyptus globulus* EO against head lice in both *in vitro* and *in vivo* tests. *In vitro* experiment used a filter paper contact method to evaluate the pediculicidal activity of the shampoo at 0.002, 0.003 and 0.006 ml/cm^2 doses (per unit area of petri dish plate) on nymphs and adults of head lice. *In vivo* trial, the infested children were treated with the shampoo. The results showed either cured or not cured of pediculosis. The main results showed LC_{50} values against nymphs for *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo were 0.00001 and 0.00004 ml/cm^2, respectively. Those actively against the adults which were 0.7 and 0.9 ml/cm^2, respectively. *In vivo* test revealed that *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo were the most effective pediculicide, showing 100% cure rate after the 2nd application, much more effective than the tested chemical pediculicide. It concluded that these shampoos can be highly affected pediculicide alternatives and safe for treating humans.

Keywords: *Pediculus humanus capitis*, Herbal shampoo, *Garcinia dulcis*, *Citrus aurantium* EO, *Eucalyptus globulus* EO.

Introduction

Head lice infestation or pediculosis is caused by head louse, *Pediculus humanus capitis* De Geer (*P. humanus capitis*): (Phthiraptera). It is one of the most common medical insects infesting humans worldwide. It affects children the most and each year approximately five million children are newly infested with head lice (Bowles *et al*., 2017; National Association of School Nurses,

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2018). Permethrin, malathion, cabaryl, lindane are neurotoxic synthetic insecticides used as pediculicide for head lice treatment worldwide. Unfortunately, head lice resistance to neurotoxic pediculicides have occurred in several parts of the world (Centers for Disease Control and Prevention, 2017; Devore and Schutze, 2015; Doroodgar *et al*., 2014; Eroglu *et al*., 2016). Alternative pediculicides for head lice treatment are critically needed. Recently, alternative pediculicides from plants or herbs have attracted the attention of researchers as new options for head lice treatment because of their low mammalian toxicity and high safety for children. Their mode of action are not neurotoxic, so the possibility that head lice will develop a resistance to them is low (Strycharz *et al*., 2014; Watcharawit and Soonwera, 2013). Herbal shampoos from *Averrhoa bilimbi*, *Clitoria ternatea*, *Myristica fragrans*, *Plectranthus amboincus*, *Tacca chantrieri*, *Zingiber cassumunar* and *Zanthoxylum limonella* have been shown to exhibit strong pediculicidal activities (Watcharawit and Soonwera, 2013). Essential oils (EOs) from *Geranium maculatum*, *Myrcianthes cisplatensis*, *Eucalyptus cinerea*, *Eucalyptus viminalis* and *Eucalyptus saligna* also showed pediculicidal activities (Gallardo *et al*., 2012; Toloza *et al*., 2006) and lotions based on lavender, peppermint and eucalyptus EOs exhibited strong pediculicidal activities as well (Audino *et al*., 2007).

In 2013, pediculosis was at a high level with more than 50% of Thai kindergarten children (3-5 years old) and primary school children (6-12 years old) got infested, especially the children in the rural area of Thailand. Normally, the first option for head lice treatment is to use a synthetic chemical to control them. Unfortunately, most chemical pediculicides in the market in Thailand are neurotoxic pediculicides and several of these have lost their efficacy due to increased resistance (Watcharawit and Soonwera, 2013). Moreover, the highly toxic effects of chemical pediculicides on children have been recorded in several parts of the world. Insecticidal pediculicides are toxic to children’s respiratory and nervous systems (Eisenhower and Farrington, 2012; Leung *et al*., 2005). Infested children in Thailand desperately need effective and safe pedicilicides for their head lice treatment.

Therefore, we were determined to investigate the pediculicidal activity of a shampoo made from *Garcinia dulcis* (Roxb.) (*G*. *dulcis*) and to compare its efficacy with those of carbaryl shampoo, permethrin shampoo and drinking water. *G*. *dulcis* plant, called “Maphut” in Thailand, belongs to the family Clusiaceae (Guttiferae). This plant species is edible and also used as a medicinal plant in Thailand. The major compounds of *G*. *dulcis* extract are Dulcisxanthone G and 1,3,6-trihydroxy-2-(2,3-dihy-droxy-3-methylbutyl)-7-methoxt-8-(3 methyl-2-butenyl) xanthone (Ripe fruit). All parts of *G. dulcis* has long been used in traditional Thai medicine with efficacies such as antioxidant, antiviral, anticancer, anti-inflammatory, antibacterial, hypocholesterolemic, tonic and radical scavenging. Mature fruits of *G. dulcis* are also made into soft drink, jam and fruit paste (Deachathai *et al*., 2005; Lim, 2011; Lamai *et al*., 2013; Tuansulong *et al*., 2011).

The augmenting EOs tested in this study were from *Citrus aurantium* L. (*C. aurantium*) and *Eucalyptus globulus* Labill (*E. globulus*) belonging to the family Rutaceae and Myrtaceae. The major compounds of *C. aurantium* EO were limonene, 4-terpineol, linalool, l-linalool, (+)-auraptenal, α-pinene, β-myrcene, acetic-acid, d-limonene, β-pinenegamma-terpinene, and linalyl acetate. The major compounds of *E. globulus* EO were 1,8-cineole, α-pinene, limonene, terpineol, guaaiacol, globulol, α-phellandrene, tannin, aromadendrene, pinocarvon, pinocarveol, eucalyptin, and rutin (Barbosa *et al*., 2016; Suryawanshi, 2011). These plants are cultivated throughout Southeast Asia including Thailand. EOs from *C. aurantium* and *E. globulus* have long been used as traditional Thai medicine for cough, dizziness, cramping, flatulence, indigestion (Sinthusart, 2015; Tracy and Kingston, 2007). Their efficacies also include analgesic, antifungal, antineuralgic, antirheumatic, antiseptic, anti-parasitic, anti-anxiety and sedative (Barbosa *et al*., 2016; Sanei-Dehkordi *et al*., 2016; Suryawanshi, 2011). In the present study, the efficacy of an herbal shampoo made from *G. dulcis* added with either *C. aurantium* EO or *E. globulus* EO against head lice were investigated.

Materials and methods

Fruit collection and preparation of essential oils and herbal shampoo

Mature fruits of *G. dulcis* were collected from Nakhonratchasima province in the North-eastern part of Thailand during May-June 2016. They were positively identified by a taxonomist at the Faculty of Agricultural Technology, KMITL, Thailand. Plant essential oils (EOs) from fresh fruit of *C. aurantium* and fresh leaves of *E. globulus* were extracted by water distillation method. The collected fruits of *G. dulcis* and essential oils from *C. aurantium* and *E. globulus* were used to prepare 3 formulations of herbal shampoo at 10% concentration by a medical plant scientist at KMITL as *G. dulcis* shampoo (10% (v/v) aqueous crude extract of *G. dulcis* fruits + 89 % water + 1% emulsifier), *G. dulcis* + *E. globulus* EO shampoo (10% (v/v) aqueous crude extract of *G. dulcis* fruits + 10% *E. globulus* EO + 79% water + 1% emulsifier) and *G. dulcis* + *C. auratium* EO shampoo (10% (v/v) aqueous crude extract of *G. dulcis* fruits + 10% *C. auratium* EO + 79 % water + 1% emulsifier). All plant shampoos were stored in the laboratory at 27±5 °C and 70±5% RH. Carbaryl and permethrin shampoos were used as positive controls and drinking water was used as negative control.

Insecticidal shampoos and drinking water

- Carbaryl shampoo (Hafif shampoo®, 0.6% *w/v* carbaryl) was purchased from IDS Manufacturing Co. Ltd., Pathumthani province, Thailand.
- Permethrin shampoo (Scully shampoo®, 0.5% *w/v* permethrin) was purchased from Sherwood Chemical Manufacturing Co. Ltd., Chacheangsao province, Thailand.
- Drinking water (Singha®) was manufactured by Boon Rawd Brewery Co. Ltd., 999 Samsen Rd, Dusit, Bangkok, Thailand.

Collection of head lice

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). All head lice (3rd nymphs and adults) were collected from the heads of 50 infested subjects who were students and parents of some students at several primary schools in Samutprakarn province, Thailand. Nymphs and adults of head lice were carefully removed from the teeth of lice combs and separated into clean insect boxes (18.0x23.0x5.5 cm). Each stage of head lice was separated under a stereomicroscope within 15-20 min after the collection.

Contact toxicity bioassay

We used a filter paper contact method to evaluate the pediculicidal activity of the tested shampoos. This method was adapted from the method in Watcharawit and Soonwera (2013). Each tested herbal and chemical shampoo at 0.002, 0.003 and 0.006 ml/cm^2 doses and the negative control were applied to a filter paper (Whatman® No1, 4.8 cm diameter) and after having been left to dry for 30s, each filter paper was placed at the bottom of a petri dish (5.0 cm diameter). Ten nymphs or 10 adults of head lice were put and left on the shampoo-treated filter paper for one hour. The mortality rates of nymphs and adults were recorded at 10, 30 and 60 minutes. The criterion for mortality of head louse was defined as absolutely no movement of external or internal structures of head lice's body (Watcharawit and Soonwera, 2013). The criterion for effective pediculicidal activity was defined as an LT_{50} value of < 1.0

minute. Each test was performed in 10 replicates with simultaneous negative control. The data means were compared by Duncan's multiple range test.Statistical significance was set at $p<0.05$. LT_{50} and LC_{50} values were calculated by Probit analysis. The mortality percentage was calculated using the following formula:

$$\%\ \text{Mortality} = \frac{\text{Number of dead head lice}}{\text{Total number of head lice}} \times 100$$

In vivo test

A total of 150 infested schoolchildren between the ages of 5 to 12 years from three primary schools in Samutprakarn province, Thailand, were selected to participate in the *in vivo* test. The criterion for pediculosis was defined as the presence of at least one live nymph or adult or egg. All infested schoolchildren in this study were allowed to use only a lice comb for head lice treatment during the experimental period. They had not been treated with any pediculicides before. The 150 infested schoolchildren were randomly separated into four groups (10 schoolchildren per group) and treated as follows:

Group 1 was treated with *G. dulcis* shampoo;
Group 2 was treated with *G. dulcis* + *E. globulus* EO shampoo;
Group 3 was treated with *G. dulcis* + *C. aurantium* EO shampoo;
Group 4 was treated with carbaryl shampoo;
Group 5 was treated with permethrin shampoo.

The subjects in each group were treated with the corresponding shampoo by applying 15 ml of the shampoo into their wet hair and scalp, working it in for 10 minutes and then rinsing it off with clean water. The cure rate of each shampoo was recorded after the 1st application. After the 1st application on day 1, 2nd application was performed on the subjects who still had had head lice and then the cure rates for this application were recorded. The 3rd application was performed 1 day later for the subjects who still had had head lice and the cure rates were similarly recorded. Each test was replicated three times. Percentage cure rate was calculated using the following formula,

$$\%\text{Cure rate} = \frac{\text{Number of cured schoolgirls}}{\text{Total number of schoolgirls}} \times 100$$

Results

The mortality rates, LT_{50} values and LC_{50} values provided by *G. dulcis* shampoo, *G. dulcis* + *E. globulus* EO shampoo, *G. dulcis* + *C. aurantium* EO shampoo, carbaryl and permethrin shampoos at 0.002, 0.003 and 0.006 ml/cm^2 doses against nymphs of head lice are listed in Table 1. At 0.002 ml/cm^2, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo showed an LT_{50} values of 2.4 and 3.7 minutes and 100% mortality at 30 min, followed by *G. dulcis* shampoo that showed an LT_{50} value of 10.4 min and mortality ranging from 66.7-80.0% at 10 to 60 min. The LT_{50} values of carbaryl and permethrin shampoos were 9.1 and 58.5 min, respectively, while drinking water (negative control) showed no LT_{50} value. At 0.003 ml/cm^2, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO showed LT_{50} values of 1.9 and 2.2 min and 100% mortality at 30 min, while *G. dulcis* shampoo showed an LT_{50} value of 8.7 min and mortality ranging from 73.3-88.0% at 10 to 60 min. The LT_{50} values for carbaryl and permethrin shampoos were 8.2 and 55.8 min, respectively. At 0.006 ml/cm^2, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo showed an LT_{50} value of < 1.0 min and 100% mortality at 10 min. *G. dulcis* and carbaryl shampoos showed an LT_{50} values of 6.8 and < 10.0 min and 93.3 and 89.0% mortality at 60 min. Permethrin shampoo showed an LT_{50} value of 28.9 and mortality ranging from 42.0 to 73.0% (Figure 1, A). *G. dulcis* + *C. aurantium* EO shampoo was the most effective pediculicide with an LC_{50} value of 0.00001 ml/cm^2, followed by *G. dulcis* + *E. globulus* EO shampoo, *G. dulcis* shampoo, carbaryl and permethrin shampoos with LC_{50} values of 0.00004, 0.0010, 0.002, and 0.1 ml/cm^2, respectively. Drinking water showed no LC_{50} value. There were significant differences in mean mortality rates ($p<0.05$) between all of the 3 treatments. The five tested shampoos showed mortality rates ranging from 31.0 to 100%.

The mortality rates, LT_{50} values and LC_{50} values provided by the five tested shampoos and drinking water against adult head lice are listed in Table 2. At 0.002 ml/cm^2, *G. dulcis* + *C. aurantium* EO shampoo showed an LT_{50} value of 4.4 min and 96.0% mortality at 60 min. The four other shampoos showed LT_{50} values between 8.5 to 61.8 min and mortality rates ranging from 50.0 to 84.0% at 60 min. At 0.003 ml/cm^2, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo showed LT_{50} values of 1.9 and 3.3 min and 100% mortality at 60 min, followed by *G. dulcis* shampoo which showed an LT_{50} value of 11.8 min and 93.3% mortality at 60 min. The LT_{50} values for carbaryl and permethrin shampoos were 9.5 and 38.5 min, respectively. It showed mortality rates ranging from 60.0 to 77.0% at 60 min. At 0.006 ml/cm^2

concentration, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo were the most effective as pediculicide with an LT_{50} value of < 1.0 min and 100% mortality at 10 min, followed by *G. dulcis* shampoo that showed an LT_{50} value of 9.8 min and 93.3% mortality at 60 min (Figure 1, B). The LT_{50} values for carbaryl and permethrin shampoos were 6.7 and 35.2 min, respectively. They showed mortality rates between 62.0 to 86.0% at 60 minutes. *G. dulcis* + *C. aurantium* EO shampoo was the most effective as pediculicide with an LC_{50} value of 0.002 ml/cm^2, followed by *G. dulcis* + *E. globulus* EO shampoo, *G. dulcis* shampoo, carbaryl and permethrin shampoos with LC_{50} values of 0.004, 0.009, 0.02 and 0.2 ml/cm^2, respectively. Drinking water showed no LC_{50} and LT_{50} values. The mean mortality rates shown between each pair of shampoos were statistically significantly different (p<0.05). The tested shampoos showed mortality rates between 30.0 to 100%.

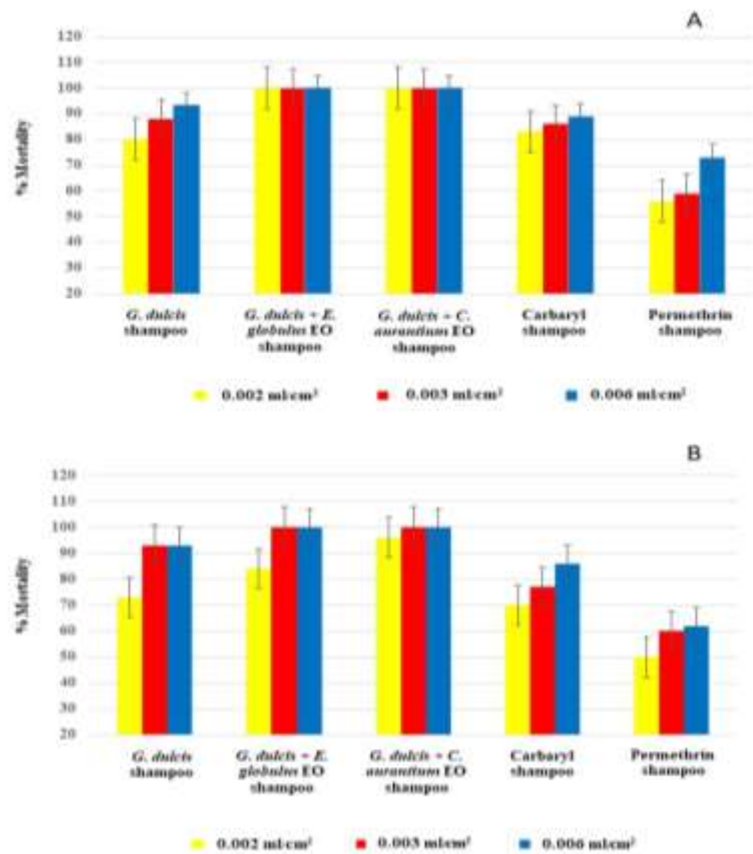

Figure 1. Percentage mortality of *P. humanus capitis* nymphs (A) and adults (B) caused by three herbal shampoos and insecticidal shampoos.

Table 1. Mortality rates and LT_{50} and LC_{50}values of five tested shampoos against *P. humanus capitis* nymphs at three concentrations at 10, 30 and 60 minutes post-exposure

Treatment	Dose (ml/cm^2)	Mortality (%)[a] ± SD			LT_{50} (min)	95% Confidence Limit	
		10 min	30 min	60 min		LCL	UCL
G. dulcis shampoo	0.002	66.7 ±11.5e	73.3 ±11.5d	80.0 ±20.0c	10.4	8.3	12.3
	0.003	73.3 ±11.5d	88.0 ±26.8c	88.0 ±26.8bc	8.7	5.6	10.2
	0.006	80.0 ±28.3c	93.3 ±11.5b	93.3 ±11.5b	6.8	4.6	8.8
LC_{50} value = 0.0010 ml/cm^2 (at 10 min)							
G. dulcis + *E. globulus* EO shampoo	0.002	78.0 ±15.0cd	100a	100a	3.7	2.0	4.7
	0.003	96.0 ±4.8b	100a	100a	2.2	1.1	3.8
	0.006	100a	100a	100a	0.3	0.02	1.4
LC_{50} value = 0.00004 ml/cm^2 (at 10 min)							
G. dulcis + *C. auratium* EO shampoo	0.002	92.0 ±11.0b	100a	100a	2.4	1.3	3.5
	0.003	96.0 ±8.9b	100a	100a	1.9	0.9	2.6
	0.006	100a	100a	100a	0.2	0.01	1.1
LC_{50} value = 0.00001 ml/cm^2 (at 10 min)							
Carbaryl shampoo	0.002	68.0 ±14.8de	77.0 ±8.2d	83.0 ±4.8d	9.1	7.5	11.1
	0.003	75.0 ± 12.7cd	78.0 ± 7.9d	86.0 ±5.2c	8.2	6.3	10.7
	0.006	82.0 ±9.2bc	84.0 ±9.7c	89.0 ± 7.9bc	6.3	4.3	7.21
LC_{50} value = 0.002 ml/cm^2 (at 10 min)							
Permethrin shampoo	0.002	31.0 ±8.0f	44.0 ±5.2e	56.0 ±9.7e	58.5	49.3	65.7
	0.003	32.0 ±8.2f	48.0 ± 9.2e	59.0 ±9.9e	55.8	47.3	61.5
	0.006	42.0 ±11.9f	70.0 ±6.7de	73.0 ±6.8de	28.9	22.7	31.3
LC_{50} value = 0.10 ml/cm^2 (at 10 min)							
Drinking water	0.002	0g	0f	0f	NA	NA	NA
	0.003	0g	0f	0f	NA	NA	NA
	0.006	0g	0f	0f	NA	NA	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} = 50% lethal time; *LC_{50}* = 50% lethal concentration; *UCL* is upper confidence limit; *LCL* is lower confidence limit; NA means not computed from this Probit analysis.

Table 2. Mortality rates and LT_{50} and LC_{50}values of five tested shampoos against *P. humanus capitis* adults at three concentrations at 10, 30 and 60 minutes post-exposure

Treatment	Does (ml/cm^2)	Mortality (%) [a] ± SD			LT_{50} (min)	95% Confidence Limit	
		10 min	30 min	60 min		LCL	UCL
G. dulcis shampoo	0.002	60.0 ±20.0e	66.7 ±11.5de	73.3 ±11.5d	12.4	9.7	14.5
	0.003	60.0 ±20.0e	66.7 ±11.5de	93.3 ±11.5b	11.8	9.2	13.9
	0.006	66.7 ±11.5e	73.3 ±11.5d	93.3 ±11.5b	9.8	7.5	12.6
LC_{50} value = 0.009 ml/cm^2 (at 10 min)							
G. dulcis + E. globulus EO shampoo	0.002	78.0 ±7.8d	80.0 ±6.7c	84.0 ± 8.9c	8.5	6.7	10.3
	0.003	82.0 ±7.9c	94.0 ±5.2b	100a	3.3	2.5	4.8
	0.006	100a	100a	100a	0.9	0.5	1.6
LC_{50} value = 0.004 ml/cm^2 (at 10 min)							
G. dulcis + C. auratium EO shampoo	0.002	96.0 ±8.9b	96.0 ±8.9ab	96.0 ±8.9b	4.4	3.8	4.7
	0.003	96.0 ±8.9b	100a	100a	1.9	0.9	2.6
	0.006	100a	100a	100a	0.7	0.5	1.1
LC_{50} value = 0.002 ml/cm^2 (at 10 min)							
Carbaryl shampoo	0.002	52.0 ±7.9ef	67.0 ±6.8de	70.0 ±14.1d	10.1	9.3	13.9
	0.003	69.0 ±8.6de	75.0 ±8.5cd	77.0 ±4.8cd	9.5	7.8	12.7
	0.006	72.0 ±7.9d	81.0 ±6.7c	86.0 ±10.3c	6.7	4.7	10.3
LC_{50} value = 0.02 ml/cm^2 (at 10 min)							
Permetrin shampoo	0.002	30.0 ±7.1f	42.0 ±8.4f	50.0 ± 9.3e	61.8	57.3	63.7
	0.003	31.0 ±6.7f	46.0 ±7.5f	60.0 ± 8.5e	38.5	35.2	41.4
	0.006	41.0 ±8.7f	53.0 ±8.2e	62.0 ±7.9e	35.2	33.7	37.9
LC_{50} value = 0.2 ml/cm^2 (at 10 min)							
Drinking water	0.002	0g	0g	0f	NA	NA	NA
	0.003	0g	0g	0f	NA	NA	NA
	0.006	0g	0g	0f	NA	NA	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} = 50% lethal time; *LC_{50}* = 50% lethal concentration; *UCL* is upper confidence limit; *LCL* is lower confidence limit; NA means not computed from this Probit analysis.

The cure rates of the school children after the 1st, 2nd and 3rd applications are listed in Figure 2. After the 1st application, *G. dulcis* + *C. aurantium* EO shampoo showed a cure rate of 90.6%, followed by *G. dulcis* + *E. globulus* EO shampoo, *G. dulcis* shampoo, carbaryl and permethrin shampoos with cure rates of 84.5, 70.0, 73.3 and 16.7%, respectively. After the 2nd application, *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo were shown to be the most effective as pediculicide with 100% cure rate, followed by *G. dulcis*, carbaryl and permethrin shampoos with cure rates of 75.3, 83.3 and 23.3%, respectively. The cure rates for *G. dulcis* + *C. aurantium* EO shampoo and *G. dulcis* + *E. globulus* EO shampoo, *G. dulcis* shampoo, carbaryl and permethrin shampoos after the 3rd application was 100, 100, 78.5, 90.0 and 27.8%, respectively. There were significant differences in the mean cure rates and in the mean pediculosis rates ($p<0.05$) between the 5 tested shampoos. The 3 tested herbal shampoos showed cure rates between 70.0 to 100%. Furthermore, after the 1st, 2nd or 3rd applications, none of the schoolchildren experienced any negative side effects such as red spots on the scalp and neck, burning sensation or irritation of the scalp. On the other hand, carbaryl and permethrin shampoos caused some side effects to schoolchildren such as red spots on the scalp and neck (6.6%), burning sensation (6.6%) and irritation (6.6%) of the scalp (Table 3).

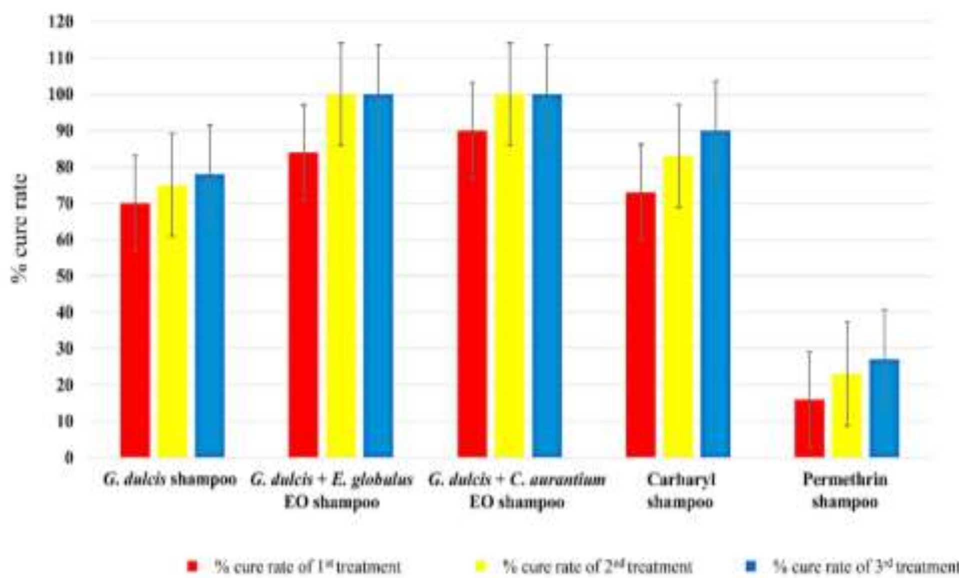

Figure 2. Cure rates of school girls the1st, 2nd and 3rd applications

Table 3. Side effects among schoolchildren after the 1st, 2nd and 3rd applications

Tested shampoo	Application	Side effects		
		Red spot	Burning sensation	Irritation
G. dulcis shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
G. dulcis + *E. globulus* EO shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
G. dulcis + *C. auratium* EO shampoo	1st	no	no	no
	2nd	no	no	no
	3rd	no	no	no
Carbaryl shampoo	1st	yes (6.6%)	yes (6.6%)	yes (6.6%)
	2nd	no	yes (6.6%)	no
	3rd	yes (6.6%)	no	no
Permethrin shampoo	1st	yes (6.6%)	yes (6.6%)	yes (6.6%)
	2nd	yes (6.6%)	yes (6.6%)	no
	3rd	no	yes (6.6%)	no

Discussion

As results, it showed that *G. dulcis* with *C. aurantium* EO and *G. dulcis* with *E. globulus* EO shampoos had the highest insectidal activity against head lice with 100% mortality of nymphs and adults at 0.006 ml/cm^2 dose and LT_{50} value of < 1.0 min and LC_{50} value of <0.00001 and < 1.0 ml/cm^2, respectively. They showed 100% cure rate after the 2nd application. These two shampoos have a high potential for killing head lice and no negative side effects after application. Therefore, *G. dulcis* with *C. aurantium* EO and *G. dulcis* with *E. globulus* EO shampoos are suitable for use as alternative pediculicides for head lice treatment of infested children. They are safe and highly effective pediculicides. The extract from *G.dulcis* fruit had 19 constituents.The major constituents were monoterpenoids such as linalool, α-terpineol and hexadecanoic acid (Lim, 2011). Linalool blocks the respiratory system of insects (Di Campli *et al*., 2012). This report is in agreement with a report by Beier *et al.* (2014) that linalool in basil oil was active against tephritid fruit flies (*Ceratitis capitate*

(Wiedemam)) and *Bactrocera dorsalis* as well as deterred the ovipositioning and egg hatching of housefly, *Musca domestica* L. Candy *et al*. (2018) also reported that linalool in lavender oil showed the best adulticidal activity against head lice. Extract of *G. dulcis* roots is commonly used as antipyretic and anti-toxic as well as detoxification (Deachathai *et al*., 2005; Lamai *et al*., 2013; Lim, 2011). Traditional medicine of Thailand and Indonesia has used the fruits, seeds and leaves of *G. dulcis* to treat several human diseases such as a relief expectorant for coughs and a medicine for scurvy, hydrocele, lymphatitis and parotitis. The extract of *G. dulcis* fruits and leaves is used as an anti-HIV, antiviral, antibacterial, anti-inflammatory, antitumor, anticancer and antioxidant agent (Abu Bakar *et al*., 2015; Hutadilok-Towatana *et al*., 2007; Lim, 2011; Lamai *et al*., 2013; Tuansulong *et al*., 2011).

Detailed descriptions of the aromatic compounds in the EO extracted from *C*. *aurantium* and *E. globulus* were found to be the major monoterpenes components were provided by Suryawanshi (2011), Sanei-Dehkordi *et al*. (2016) and Barbosa *et al*. (2016) EO from *C*. *aurantium* peel contains limonene, α-pinene, flavonoids and triterpenes.The principal compounds found in EO from *E*. *globulus* leaves are 1,8-cineole, α-pinene, limonene and terpineol. Similar results were found by Sanei-Dehkordi *et al*. (2016) who observed that plant EO from *C.aurantium* showed the highest activity against larvae of *Anopheles stephensi*. Furthermore, Badawy *et al*. (2017) studied the larvicidal and fumigant toxicity of *Citrus reticulate* and *Citrus sinensis* against the mosquito *Culex pipiens* and attributed the toxicity to inhibition of acetylcholinesterase enzyme (AChE) of insects. Similarly, 1,8-cineole from *E*. *globulus* EO exhibited high toxicity against head lice (Barbosa *et al*., 2016; Toloza *et al*., 2010). Some researchers indicated that monoterpenes components augment the inhibitory effect on AChE because of the presence of the double bond of the carbonyl group (Dambolena *et al*., 2016).

There are many other herbal products such as those from *Piper retrofactum*, *Acorus calamus*, *Phyllanthus emblica* and *Zanthoxylum limonella* that showed high pediculicidal activity against head lice (Watcharawit and Soonwera, 2013). Audino *et al*. (2007) reported that lotions containing eucalyptus, peppermint and lavender EOs showed high mortality rates against head lice. Commercial products based on grapefruit, bergamot, clove and neem also showed high pediculicidal activity for head lice treatment (Abdel-Ghaffar *et al*., 2016).

The carbaryl shampoo tested in this study is a common pediculicide in Thailand for treating head lice. Its pediculicidal activity was much lower than *G*. *dulcis* with *C*. *aurantium* EO and *G*. *dulcis* with *E. globulus* EO shampoos, but it has serious side effects and toxicity that have been reported in several countries. This insecticidal shampoo is toxic to infested children, especially to children younger than 5 years of age and caused red spot, burning sensation and irritation after they were treated with it. It is highly toxic to children's nervous system (Eisenhower and Farrington, 2012; Wadowski *et al*., 2015). Moreover, the efficacy of carbaryl shampoo against head lice has decreased globally due to resistance. Head lice resistance to carbaryl shampoo has been reported in several countries such as Australia, UK, and USA (Durand *et al*., 2012; Eisenhower and Farrington, 2012). In the same vein, even though permethrin shampoo caused 30-75% mortality of nymphs and adults of head lice and showed 16.7-27.8% of cure rate, its pediculicidal activity was much lower than *G*. *dulcis* with *C*. *aurantium* EO and *G*. *dulcis* with *E. globulus* EO shampoos. Moreover, it caused red spot, burning sensation and irritation to school children after they were treated with it. The reason that permethrin shampoo showed a low efficacy for head lice treatment may be attributable to head lice resistance. Head lice resistance to permethrin shampoo has been reported in Europe (United Kingdom, and Denmark), the Middle East (Israel), North America (United States), South America (Argentina), Asia (Japan), and Australia (Durand *et al*., 2012; Ko and Elston, 2004; West, 2004). Permethrin shampoo has been a common and preferred shampoo for infested Thai children especially for urban children because it usually caused rapid mortality of head lice. It is toxic to head lice's nervous system, destroying the nerve cells and causing head lice mortality (Cueto *et al*., 2008; Eisenhower and Farrington, 2012). Unfortunately, the toxicity of permethrin shampoo to children and head lice resistance to permethrin shampoo that has been recorded in several countries were high. The side effects of permethrin shampoo were reported to be itching, rash and burning of children's scalp and corneal damage of children's eyes (Allen and Cox, 2018; American Academy of Pediatrics, 2017; Wadowski *et al*., 2015). Some of these effects were observed in our study as well.

In contrast, *G*. *dulcis* is an edible plant, commonly consumed in Thailand. It is also used as a medicinal plant in traditional Thai medicine. *G. dulcis* added with *C. aurantium* EO and *E. globulus* EO shampoo exhibited high efficacy as herbal pediculicide for head lice treatment and no side effects to schoolchildren. It is a safe and highly effective pediculicide. These shampoos are suitable for use as alternative herbal pediculicide for head lice treatment, especially for infested children in the rural areas of Thailand and may be a good and safe herbal pediculicide for children all over Southeast-Asia.

Our suggestion for human head lice eradication is that parents and teachers should treat infested schoolchildren with *G. dulcis* + *C. aurantium* EO or *G*. *dulcis* + *E. globulus* EO shampoos by applying 15-20 ml of the shampoo into their wet hair and scalp, working it in for 10-15 minutes, and then rinsing it off with clean water for at least 3 times in a week for a month.

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).

Acknowledgments

This work was supported by The National Research Council of Thailand (NRCT) (Grant for the Doctoral Degree Student Fly 2016) and the Faculty of Agricultural Technology, KMITL (Grant No. 2559-01-04-013), Bangkok, Thailand. We are grateful to all primary school students who were the test subjects and the teachers of the 5 primary schools in Samutprakarn province for their participation in the in-vivo and in-vitro tests and Mr. Pratana Kangsadal, the KMITL Proofreader, for reviewing and giving comments on the manuscript.

References

Abdel-Ghaffar, F., Abdel-Aty, M., Rizk, I., Al-Quraishy, S., Semmler, M., Gestmann, F. and Hoff, N. P. (2016). Head lice in progress: what could/should be done-a report on an in vivo and in vitro field study. Parasitology Research 115:4245-4249.

Abu Bakar, M. F., Ahmad, N. E., Suleiman, M., Rahmat, A. and Isha, A. (2015). *Garcinia dulcis* fruit extract induced cytotoxicity and apoptosis in HepG2 liver cancer cell line. BioMed Research International 2015:916902. doi: 10.1155/2015/916902.

Allen, H. and Cox, J. (2018). Permethrin cream (Lyclear). 20 March 2018. Retrieved from http://patient.info/medicine/permethrin-cream-lyclear.

American Academy of Pediatrics. (2017). Head lice: what parents need to know. 20 December 2017. Retrived from https://www.headltychildren.org.

Audino, P. G., Vassena, C., Zerba, E. and Picollo, M. (2007). Effectiveness of lotions based on essential oils from aromatic plants against permethrin resistant *Pediculus humunus capitis*. Archives of Dermatological Research 299:389-392.

Badawy, M. E. I., Taktak, N. E. M. and El-Aswad, A. F. (2017). Chemical composition of the essential oils isolated from peel of three citrus species and their mosquitocidal activity against *Culex pipiens*. Natural Product Research 10:1-6.

Barbosa, L. C., Filomeno, C. A. and Teixeira, R. R. (2016). Chemical variability and biological activities of *Eucalyptus* spp. essential oils. Molecules 21:E1671.

Beier, R. C., Byrd, J. A., Kubena, L. F., Hume, M. E., McReynolds, J. L., Anderson, R. C. and Nisbet, D. J. (2014). Evaluation of linalool, a natural antimicrobial and insecticidal essential oil from basil: effects on poultry. Poultry Science 93:267-272.

Bowles, V. M., Yoon, K. S., Barker, S. C., Tran, C., Rhodes, C. and Clark, M. J. (2017). Ovicidal efficacy of abametapir against eggs of human head and body lice (Anoplura: Pediculidae). Journal of Medical Entomology 54:167-172.

Candy, K., Nicolas, P., Andriantsoanirina, V., Izri, A. and Durand, R. (2018). In vitro efficacy of five essential oils against *Pediculus humanus capitis*. Parasitology Research 117:603-609.

Centers for Disease Control and Prevention (2017). Head lice treatment. 25 December 2017. Retrived from http://www.cdc.gov/dodx/contact.html.

Cueto, G. M., Zerba, E. N. and Picollo, M. I. (2008). Evidence of pyrethroid resistance in eggs of *Pediculus humunus capitis* (Phthiraptera: Pediculidae) from Argentina.Journal of Medical Entomology 45:693-697.

Dambolena, J. S., Zunino, M. P., Herrera, J. M., Pizzolitto, R. P., Areco, V. A. and Zygadlo, J. A. (2016). Terpenes: natural products for controlling insects of importance to human health-a structure- activity relationship study. Psyche. A Journal of Entomology 2016:4595823. http://dx.doi.org/10.1155/2016/4595823.

Deachathai, S., Mahabusarakam, W., Phongpaichit, S. and Taylor, W. C. (2005). Phenolic compounds from the fruit of *Garcinia dulcis*. Phytochemistry 66:2368-2375.

Devore, C. D. and Schutze, G. E. (2015). Council on school health and committee on infectious diseases. Journal of Pediatrics 135:e1355-65.

Di Campli, E., Di Bartolomeo, S., Delli Pizzi, P., Di Giulio, M., Grande, R., Nostro, A. and Cellini, L. (2012). Activity of tea tree oil and nerolidol alone or in combination against *Pediculus capitis* (head lice) and its eggs. Parasitology Research 111:1985-1992.

Doroodgar, A., Sadr, F., Doroodgar, M., Doroodgar, M. and Sayyah, M. (2014). Examining the prevalence rate of *Pediculus capitis* infestation according to sex and social factors in primary school children. Asian Pacific Journal of Tropical Disease 4:25–29.

Durand, R., Bouvresse, S., Berdjane, Z., Izri, A., Chosidow, O. and Clark, J. M. (2012). Insecticide resistance in head lice:clinical, parasitological and genetic aspects.Clinical Microbiology and Infection 18:338-344.

Eisenhower, C. and Farrington, E. A. (2012). Advancements in the treatment of head lice in pediatrics. Journal of Pediatric Health Care 26:451-461.

Eroglu, F., Basaran, U., Kurklu, C. G., Yuceer, M., Yalcinturk, R. G., Tanriverdi, M., Dagli, E. I. and Koltas, I. S.(2016). Pediculosis capitis is a growing neglected infestation due to migration in Southeast Turkey. Parasitology Research 115:2397-2401.

Gallardo, A., Picollo, M. I., González-Audino, P. and Mougabure-Cueto, G. (2012). Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). Journal of Medical Entomology 49:332-335.

Hutadilok-Towatana, N., Kongkachuay, S. and Mahabusarakam, W. (2007). Inhibition of human lipoprotein oxidation by morelloflavone and camboginol from *Garcinia dulcis*. Natural Product Research 21:655-662.

Ko, C. J. and Elston, D. M. (2004). Pediculosis. Journal of the American Academy of Dermatology 50:1-12.

Lamai, J., Mahabusarakam, W., Ratithammatorn, T. and Hiranyachattada, S. (2013). Effects of morelloflavone from *Garcinia dulcis* on vasorelaxation of isolated rat thoracic aorta. Journal of Physiological and Biomedical Sciences 26:13-17.

Leung, A. K. C., Fong, J. H. S. and Pinto-Rojas, A. (2005). Pediculosis capitis. Journal of Pediatric Health Care 19:369-373.

Lim, T. K. (2011). Edible medicinal and non-medicinal plants. Fruits 2:35-40.

National Association of School Nurses. (2018). Head lice management in the school setting. 20 January 2018. Retrived from https://schoolnursenet.nasn.org.

Sanei-Dehkordi, A., Sedaghat, M. M., Vatandoost, H. and Abai, M. R. (2016). Chemical compositions of the peel essential oil of *Citrus aurantium* and its natural larvicidal

activity against the malaria vector *Anopheles stephensi* (Diptera: Culicidae) in comparison with *Citrus paradisi*. Journal of Arthropod-Borne Diseases 10:577–585.
Sinthusart, U. (2015). Herbs of Chao-Krom-Poe Dispensary. 5th ed. Bangkok: Thailand. pp. 356.
Strycharz, J. P., Lao, A. R., Alves, A. M. and Clark, J. M. (2014). Ovicidal response of NYDA formulations on the human head louse (Anoplura: Pediculidae) using a hair tuft bioassay. Journal of Medical Entomology 49:336-342.
Suryawanshi, J. A. S. (2011). An overview of *Citrus aurantium* used in treatment of various diseases. African Journal of Plant Science 5:390-395.
Toloza, A. C., Zygadlo, J., Cueto, G. M., Biurrun, F., Zerba, E. and Picollo, M. I. (2006). Fumigant and repellent properties of essential oils and component compounds against permethrin resistant *Pediculus humunus capitis* (Anoplura: Pediculidae) from Argentina. Journal of Medical Entomology 43:889-895.
Toloza, A. C., Lucía, A., Zerba, E., Masuh, H. and Picollo, M. I. (2010). Eucalyptus essential oil toxicity against permethrin-resistant *Pediculus humanus capitis* (Phthiraptera: Pediculidae). Parasitology Research 106:409-414.
Tracy, T. S. and Kingston, R. L. (2007). Herbal products: toxicology and clinical pharmacology. Totowa: New Jersey. pp. 288.
Tuansulong, K. A., Hutadilok-Towatana, N., Mahabusarakam, W., Pinkaew, D. and Fujise, K. (2011). Morelloflavone from *Garcinia dulcis* as a novel biflavonoid inhibitor of HMG-CoA reductase. Phytotherapy Research 25:424-428.
Wadowski, L., Balasuriya, L., Price, H.N. and O'Haver, J. (2015). Lice update: new solutions to an old problem. Clinics in Dermatology 33:347-354.
Watcharawit, R. and Soonwera, M. (2013). Pediculicidal effect of herbal shampoo against *Pediculus humunus capitis* in vitro. Tropical Biomedicine 30:315-324.
West, D. P. (2004). Head lice treatment costs and the impact on managed care. The American Journal of Managed Care 10:S277-82.

(Received: 18 April 2018, accepted: 5 May 2018)