Asian Pacific Journal of Tropical Biomedicine

Journal homepage: www.apjtb.org

doi: 10.4103/2221-1691.225614

New Thai herbal shampoos as pediculicides for killing head louse, *Pediculus humanus capitis* De Geer (Phthiraptera)

Sirawut Sittichok, Orawan Wongnet, Mayura Soonwera✉

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand

ARTICLE INFO

Article history:
Received 31 October 2017
Revision 15 December 2017
Accepted 10 January 2018
Available online 16 February 2018

Keywords:
Pediculus humanus capitis
Thai herbal shampoo
Cratoxylum formosum + eucalyptus essential oil shampoo
Ovicicide
Nymphicide
Adulticide

ABSTRACT

Objective: To evaluate the efficacy of Thai herbal shampoos [*Cartoxylum formosum* (*C. formosum*) + eucalyptus essential oil (EO), *C. formosum* + citrus EO, *Solanum trilobatum* + eucalyptus EO, *Solanum trilobatum* + citrus EO, *Moringa oleifera* + eucalyptus EO and *Moringa oleifera* + citrus EO] for killing all stages of *Pediculus humanus capitis* (Phthiraptera). **Methods:** A filter paper contact method was applied with three concentrations (0.05, 0.10 and 0.20 mL/cm^2) of each Thai herbal shampoo as well as permethrin pediculicide (positive control) and drinking water (negative control) against eggs, nymphs and adults of *Pediculus humanus capitis*. Mortality rates of the eggs were recorded after 7 days of incubation while those of nymphs and adults were recorded after 5 minutes of contact. **Results:** All herbal shampoos at the high concentration were highly effective against nymphs and adults, but not effective against the eggs. *C. formosum* + eucalyptus EO and *C. formosum* + citrus EO shampoos at all concentrations exhibited the highest efficacy against nymphs and adults with 100% mortality rate at 5 min and LC_{50} values of 0.004 and 0.005 mL/cm^2, respectively. All formulation of *Solanum trilobatum* and *Moringa oleifera* shampoos added with eucalyptus EO showed mortality rates against nymphs at 92.0%-100.0% and 76.0%-100.0% and against adults at 84.0%-100.0% and 20.0%-32.0%, respectively. Permethrin pediculicide was not effective against the eggs, but showed 68.0%-92.0% and 28.0%-60.0% mortality rates against nymphs and adults. **Conclusions:** These results indicate that *C. formosum* + eucalyptus EO shampoo can be used as an effective nymphicide and adulticide against *Pediculus humanus capitis*.

1. Introduction

Head louse *Pediculus humanus capitis* De Geer (Pediculidae: Phthiraptera) is a small and wingless insect with the body side of 2.5-4.5 mm. It is an ectoparasitic insect that feeds only on human blood more than three times per day. The most common symptoms of head lice infestation are red spots, irritation, pruritus of the scalp, and the secondary symptoms are sleep loss, blood loss, anemia and psychological distress[1-4]. Traditionally, pediculicides based on neurotoxic insecticides (lindane, malathion, carbaryl, permethrin and

✉Corresponding author: Mayura Soonwera, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand.
Tel: 66 (0) 89-124-6455, 66 (0) 86-409-7770
Fax: 66 (0) 2-3298514
E-mail: mayura.soon@gmail.com
Foundation project: This research was supported by King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok 10520, Thailand (Grant No.2560-01-04-003) and the National Research Council of Thailand (Grant for the Doctoral Degree Student Fly 2016).

How to cite this article: Sittichok S, Wongnet O, Soonwera M. New Thai herbal shampoos as pediculicides for killing head louse, *Pediculus humanus* capitis De Geer (Phthiraptera). Asian Pac J Trop Biomed 2018; 8(2): 106-112.

phenothrin) are the first option for head lice treatment. Unfortunately, head lice resistance to neurotoxic insecticides has occurred and been reported[1-4]. Moreover, adverse effects of chemical pediculicides such as irritation and burning sensation to the scalp, ataxia, tremor and seizure have been noted[4,5]. Presently, there are considerable concerns about chemical pediculicides related to human health, long chemical persistence in the environment, food supply, water, and air. Most importantly, head lice resistance to chemical pediculicides has already developed[6-8]. Thus, new pediculicides for head lice treatment are needed. More than ten products based on plant extracts from neem, clove, henna, Zingiberacera plants, Piperaceae plants and essential oil from Zingiberacera plants, Rutaceae plants, eucalyptus, lavender, coconut and yalang-yalang have been tested as pediculicide for head lice treatment[9-15]. Pediculicides from plants have been recommended as good and safe alternative pediculicides for head lice treatment because their toxicity to human's nerve and respiratory systems is low[16,17]. Natural pediculicides from plants have been investigated for nymphicidal and adulticidal activities against head lice[11-14,15]. Moreover, natural products are effective, easily biodegradable, inexpensive and environmental friendly, having no negative effects on non-target organisms[16-18]. The chemical compounds from plant extracts and plant essential oils (EOs) exhibited an inhibition effect on acetylcholinesterase on the nervous system of insects and caused its paralysis and death[19,20].

Several papers published in 2004, 2008, 2016, and 2017 report that EOs from *Eucalyptus globulus* (*E. globulus*) leaves, *Cinnamomum aromaticum* bark, *Eugenia aromatica* flowering buds, *Pimpinella anisum* fruits, *Sesamum indicum* seeds, aerial parts of *Mentha spicata* and *Thymus vulgaris* were active against eggs and female adults of head lice[21-25]. Shampoos of 10% crude aqueous extracts of *Citrus aurantifolia* and *Citrus hystrix* fruits exhibited highly effective pediculicidal activity against head lice[10]. Mehlhorn *et al*[26] and Al-Quraishy *et al*[27] found that neem shampoo was highly toxic against eggs and adults of head lice. However, published papers related to ovicidal, nymphicidal and adulticidal acitivities of *Cratoxylum formosum* (*C. formosum*), *Solanum trilobatum* (*S. trilobatum*) and *Moringa oleifera* (*M. oleifera*) extracts against head lice are limited and many papers that reported about the Thai herbs presented in this study inform only of their therapeutic properties and health benefits but not of their pediculicidal activity against head lice[28-34]. All Thai herbs investigated in this study are also used in traditional Thai medicine for treatment of food poisoning, diarrhea, internal bleeding, cough, and pyrexia and as expectorants. The major chemical constituents of *C. formosum*, *S. trilobatum* and *M. oleifera* extracts, EOs of *E. globulus* and *Citrus sinensis* (*C. sinensis)* were phenolic compounds and monoterpenes[35-39]. Many papers have pointed out that monoterpenes cause mortality of insects by inhibiting the activity of acetylcholinesterase enzyme in the nervous system of insects[16-20]. Therefore, the objective of this study was to determine the efficacies of new Thai herbal shampoos from *C. formosum*, *S. trilobatum* and *M. oleifera* added with either eucalyptus essential oil (eucalyptus EO) or citrus essential oil (citrus EO) as well as the efficacies of permethrin pediculicide (Scully Anti-Lice Shampoo®, 0.5% w/w permethrin) and drinking water (Kaesad®) against all stages of head lice: eggs, nymphs and adults.

2. Material and methods

2.1. Six Thai herbal shampoos

Freshly picked leaves of 5-year-old *C. formosum* trees, fresh fruits of one-year-old *S. trilobatum* trees and fresh fruits of five-year-old *M. oleifera* trees were collected from Nakhon-Ratchasima province, Thailand during the summer season, April-May, of 2016. All plant specimens were positively identified by a botanical taxonomist at the Faculty of Agricultural Technology, King Mongkut's Institute Technology Ladkrabang (KMITL). Leaves and fruits of plants were cleaned, cut into small pieces, and put in a 5 L flask. One thousand grams of each plant was soaked in 2 000 mL of 95% ethyl alcohol at room temperature for 5 d. Crude extract was separated by suction filtered. Ethyl alcohol in the filtrate was evaporated with a rotary evaporator at 70 ℃ until a crude extract was achieved. Plant EOs of fresh leaves of five-year-old *E. globulus* trees (for eucalyptus EO) and fresh fruit peels of five-year-old *C. sinensis* trees (for citrus EO) were extracted by water distillation in a modified Clevenger type apparatus for 6-8 h. Water was removed from the extracts by using anhydrous sodium sulphate. All of the plant extracts and essential oils were used to prepare 6 Thai herbal shampoos at 10% concentration by a medical plant scientist at KMITL [1. *C. formosum* + eucalyptus EO shampoo (10% ethanolic extacts of *C. formosum* leaves + 10% *E. globulus* EO + water + emulsifier), 2. *C. formosum* + citrus EO shampoo (10% ethanolic extacts of *C. formosum* leaves + 10% *C. sinensis* EO + water + emulsifier), 3. *M. oleifera* + eucalyptus EO shampoo (10% ethanolic extacts of *M. oleifera* seeds + 10% *E. globulus* EO + water + emulsifier), 4. *M. oleifera* + citrus EO shampoo (10% ethanolic extacts of *M. oleifera* seeds + 10% *C. sinensis* EO + water + emulsifier), 5. *S. trilobatum* + eucalyptus EO shampoo (10% ethanolic extacts of *S. trilobatum* seeds + 10% *E. globulus* EO + water + emulsifier), 6. *S. trilobatum* + citrus EO shampoo (10% ethanolic extacts of *S. trilobatum* seeds + 10% *C. sinensis* EO + water + emulsifier)]. All plant shampoo were stored in the laboratory (25 ℃ and 70% RH). Permethrin pediculicide was used as positive control and drinking water as negative control. Permethrin shampoo (Scully Anti-Lice Shampoo®, 0.5% w/w permethrin) was manufactured by Sherwood Chemicals Manufacturing Co. Ltd, Chachoengsao province, Thailand, and drinking water (Kaesad®) was manufactured by the Faculty of Agroindustry King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. (www.kmitl.ac.th/agrind).

2.2. Approval of protocol for collection of eggs, nymphs and adults of head lice from human beings

The protocol for collection of all stages of head lice from human beings was approved by the KMITL Ethic Committee, Ladkrabang, Bangkok, Thailand with a registration number of 2560-01-04-003 in May 2016. All stages of head lice were collected from the heads of 50 severely infested subjects who were students and parents of some students at several primary schools in Ladkrabang district, Bangkok, Thailand. Their hair was cut with scissors and put in small insect boxes (4 cm×7 cm×3 cm) for egg collection. Nymphs and adults were collected by using head lice combs. Each stage of head lice was separated under a stereomicroscope within 15-20 min after the collection.

2.3. Ovicidal, nymphicidal and adulticidal activity experiments

A filter paper contact method was used to evaluate the ovicidal, nymphicidal and adulticidal activities of six Thai herbal shampoos. Permethrin pediculicide and drinking water were used as positive and negative controls, respectively. Each shampoo at concentrations of 0.05, 0.10 and 0.20 mL/cm^2 was dropped onto a 4.5-cm diameter filter paper. Ten fertile eggs/nymphs/adults of head lice were put in contact with it for 7 d (for eggs) and 5 min (for nymphs and adults). The eggs, nymphs and adults were incubated under laboratory conditions (25 ℃ and 70% RH). The control treatments with permethrin pediculicide (Scully shampoo®, 0.5% w/v permethrin) and drinking water were arranged concurrently with the treatments of Thai herbal shampoos. Each treatment was replicated five times. The mortality of eggs was recorded at 7 d and those of nymphs and adults were recorded at 5 min. The criterion for mortality of eggs was that either their operculum did not open or opened but the nymph inside was dead. The criterion for mortality of nymphs and adults was that all of their internal and external parts did not move [21,22,26].

2.4. Statistical analysis

A completely randomized design was used in this experiment. Mortality of eggs, nymphs and adults were analyzed by analysis of variance (ANOVA) and Duncan's multiple comparison with an SPSS program for windows version 22. Significant differences between treatments were determined at $P<0.05$. The percentage mortality rate of eggs, nymphs and adults were calculated by the following formula:

Mortality rate (%) = (NT/NC)×100

Where NT is the total number of dead eggs/nymphs/adults and NC is the total number of treated eggs/nymphs/adults.

3. Results

Table 1 and 2 showed the ovicidal, nymphicidal and adulticidal activities of Thai herbal shampoos at three concentrations (0.05, 0.10 and 0.20 mL/cm^2) with eucalyptus and citrus EOs supplements in terms of mortality rate and LC_{50} values against eggs at 7-day exposure and against nymphs and adults at 5-min exposure. All Thai herbal shampoos exhibited strong activity against nymphs and adults of head lice but weak or no activity against eggs. All formulations of Thai herbal shampoo added with 10% eucalyptus EO showed more nymphicidal and adulticidal activities than those added with 10% citrus EO. All Thai herbal shampoos at high concentration (0.20 mL/cm^2) effected high mortality rates. The 0.20 mL/cm^2 of shampoos from *C. formosum* added with either 10% eucalyptus EO or 10% citrus EO effected mortality rates of 24.0%, 100% and 100.0% and 12.0%, 100% and 96.0% against eggs, nymphs and adults of head lice, respectively, while the 0.20 mL/cm^2 of shampoo from *S. trilobatum* added with eucalyptus EO effected mortality rates of 12.0%, 100% and 100% against eggs, nymphs and adults of head lice, respectively. *M. oleifera* shampoo at all concentrations added either with eucalyptus EO or citrus EO effected mortality rates of 76.0%-100.0% and 68.0%-96.0% against nymphs and 20.0%-32.0% and 20.0%-30.0% against adults of head lice, respectively. On the other hand, *S. trilobatum* and *M. oleifera* shampoos added with citrus EO, permethrin pediculicide and drinking water did not show any ovicidal activity at all. Permethrin pediculide effected mortality rates of 68.0%-92.0% and 28.0%-60.0% against nymphs and adults, respectively. As expected, drinking water did not show any ovicidal, nymphicidal and adulticidal activities at all.

All Thai herbal shampoos added with 10% eucalyptus EO were highly toxic to nymphs and adults with LC_{50} values of 0.004-0.300 mL/cm^2, while those added with 10% citrus EO showed LC_{50} values of 0.005-0.400 mL/cm^2. The LC_{50} values of *C. formosum*, *S. trilobatum* and *M. oleifera* shampoos added with 10% eucalyptus EO against eggs of head lice were 33.4, 44.8 and 44.6 mL/cm^2, respectively, and the LC_{50} value of *C. formosum* shampoo added with 10% citrus EO was 43.8 mL/cm^2. The LC_{50} values of permethrin pediculicide against nymphs and adults were 0.020 and 0.040 mL/cm^2, respectively. Drinking water did not show any LC_{50} value. The relationships between mortality rate (%) and concentrations (0.05, 0.10 and 0.20 mL/cm^2) of six Thai herbal shampoos added with either 10% eucalyptus EO or 10% citrus EO against nymphs and adults of head lice showed that as concentration of Thai herbal shampoos increased from 0.05 to 0.20 mL/cm^2, the mortality rate of nymphs and adults of head lice increased. The high concentration (0.2 mL/cm^2) of all shampoo exhibited high mortality rate of all two stage of head lice. All Thai herbal shampoos added with eucalyptus EO were more effective against all head lice than those added with citrus EO.

Table 1

Mortality rates of eggs at 7 d and those of nymphs and adults of *P. humanus capitis* at 5 min after applied with six formulations of Thai herbal shampoos, permethrin pediculicide and drinking water (mean± SD).

Treatment	Concentration (mL/cm^2)	Mortality (%)		
		Egg	Nymph	Adult
C. formosum + eucalyptus EO shampoo	0.05	12.0±9.5^{b}	100^{a}	100^{a}
	0.10	16.0±6.9^{ab}	100^{a}	100^{a}
	0.20	24.0±5.7^{a}	100^{a}	100^{a}
C. formosum + citrus EO shampoo	0.05	0^{d}	88.0±8.9^{ab}	76.0±8.9^{bc}
	0.10	0^{d}	96.0±8.9^{a}	88.0±10.9^{b}
	0.20	12.0±10.9^{b}	100^{a}	96.0±8.9^{a}
M. oleifera + eucalyptus EO shampoo	0.05	4.0±3.9^{d}	76.0±8.9^{b}	20.0±5.2^{d}
	0.10	8.0±5.5^{cd}	92.0±9.5^{a}	28.0±12.8^{d}
	0.20	12.0±7.5^{b}	100^{a}	32.0±8.8^{d}
M. oleifera + citrus EO shampoo	0.05	0^{d}	68.0±10.9^{c}	20.0±8.9^{d}
	0.10	0^{d}	88.0±14.1^{ab}	22.0±9.5^{d}
	0.20	0^{d}	96.0±8.9^{a}	30.0±7.5^{d}
S. trilobatum + eucalyptus EO shampoo	0.05	4.0±2.2^{d}	92.0±8.3^{a}	84.0±6.8^{b}
	0.10	12.0±5.5^{b}	92.0±8.8^{a}	100^{a}
	0.20	12.0±4.5^{b}	100^{a}	100^{a}
S. trilobatum + citrus EO shampoo	0.05	0^{d}	84.0±10.9^{b}	80.0±7.8^{a}
	0.10	0^{d}	88.0±8.9^{b}	86.0±8.9^{b}
	0.20	0^{d}	98.0±7.9^{a}	96.0±7.8^{a}
Permethrin pediculicide (positive control)	0.05	0^{d}	68.0±9.8^{c}	28.0±7.8^{d}
	0.10	0^{d}	80.0±11.1^{b}	36.0±6.7^{d}
	0.20	0^{d}	92.0±8.5^{a}	60.0±5.8^{c}
Drinking water (negative control)	0.05	0^{d}	0^{d}	0^{e}
	0.10	0^{d}	0^{d}	0^{e}
	0.20	0^{d}	0^{d}	0^{e}

Percent mortality rates within the same column followed by the same letter are not significantly different (one-way ANOVA and Duncan's Multiple Rang test, P<0.05).

Table 2

LC_{50} values of six formulations of Thai herbal shampoos, permethrin pediculicide and drinking water at 7 d against the eggs and at 5 min against nymphs and adults of *P. humanus capitis* (mL/cm^2).

Treatment	Egg	Nymph	Adult
C. formosum + eucalyptus EO shampoo	33.4	0.004	0.005
C. formosum + citrus EO shampoo	43.8	0.005	0.006
M. oleiferas + eucalyptus EO shampoo	44.6	0.010	0.300
M. oleiferas + citrus EO shampoo	NA	0.020	0.400
S. trilobatum + eucalyptus EO shampoo	44.8	0.005	0.005
S. trilobatums + citrus EO shampoo	NA	0.006	0.007
Permethrin pediculicide (positive control)	NA	0.020	0.040
Drinking water (negative control)	NA	NA	NA

LC_{50} value = median lethal concentration; NA, not computed by Probit analysis.

4. Discussion

The nymphicidal and adulticidal activities of all formulation of *C. formosum* and *S. trilobatum* shampoos added with either 10% eucalyptus EO or 10% citrus EO at 0.20 mL/cm^2 concentration were significantly higher than permethrin pediculicide (positive control) and drinking water (negative control) (P<0.05). All Thai herbal shampoos added with 10% eucalyptus EO at 0.05 mL/cm^2 concentration were more highly toxic to nymphs and adults of head lice than those added with 10% citrus EO. The maximum nymphicidal and adulticidal activities was 0.20 mL/cm^2 from *C. formosum* shampoo added with 10% eucalyptus EO, which effected a mortality rate of 100% at 5 min against nymphs and adults and a mortality rate against head lice eggs of 24.0%. The results pointed that eucalyptus EO acted as a synergist agent in combination with *C. formosum* shampoo. Major chemical constituents of EO extracted from *E. globulus* were monoterpenoids, 1-8-cineole, α-terpinene, α-pinene, 2-β-pinene, β-myrcene, α-phellandrene, 1-isopropenyl-3-methylbenzene, γ-terpinene, (E)-pinocarveol and 1-α-terpineol[40–42]. Many papers published in 2011, 2012 and 2013 reported that monoterpenoids from plant EOs caused mortality of insects by inhibiting the activity of acetylcholinesterase enzyme in the nervous system of the insects[20,35], and monoterpenoids showed the highest activity against eggs and adults of head lice[43]. Similarly, two papers published in 2011 and 2015 reported that an anti-louse shampoo which contained neem seed extract penetrated readily into the cuticle of eggs and adults of head lice and blocked their oxygen uptake, causing mortality[26,27]. Our results showed that *C. formosum* added with eucalyptus EO shampoo effected 100% mortality rate at 5 min against nymphs and adults of head lice. These shampoo might penetrate into the cuticle of nymphs and adults and inhibited their tracheae system, causing paralysis and death. In contrast, these shampoo showed inactivity against eggs of head lice with an inhibition rate of only 24.0% at 7 days of incubation[26,27,43]. The cuticle of the eggs is very different

from those of nymphs and adults. The cuticle of the eggs is hydrophobic and impervious to water[26,27,43]. Since all Thai herbal shampoos tested in this study were aqueous formulation, they could not penetrate through the cuticle and showed low activity or inactivity against the eggs.

All formulations of Thai herbal shampoo added with citrus EO tested in this study showed lower nymphicidal and adulticidal activities than those added with eucalyptus EO and *S. trilobatum* and *M. oleifera* shampoos added with citrus EO did not show any ovicidal activity at all. Major chemical constituents of *C. sinensis* EO are limonene, α-terpinene and α-pinene[44,45]. Similarly, two papers reported that limonene from plant EOs was low in activity against eggs and adults of head lice, and it was not very toxic to acetylcholinesterase enzyme in the nervous system of the insects[19,43]. On the other hand, α-pinene from *C. sinensis* EO exhibited high toxicity against *Culex pipiens* larvae[46].

Even though published papers about ovicidal, nymphicidal and adulticidal acitivities of *C. formosum*, *S. trilobatum* and *M. oleifera* extracts against head lice are limited, but two papers published in 2009 and 2012 reported that extracts of *M. oleifera* seeds showed larvicidal, oviposition deterrent and ovicidal activities against two mosquito species, *Aedes aegypti* and *Culex quinquefasciatus*[47-49]. Extracts of *S. trilobatum* leaves exhibited larvicidal and pupicidal activities against *Aedes aegypti*, *Culex quinquefasiatus* and *Anopheles stephensi*[50] and an extract of *C. formosum* showed some therapeutic properties for humans such as antioxidant, anticancer, antimicrobial and anti-Alzheimer's disease[51,52].

Our results showed that, all formulations of *C. formosum* and *S. trilobatum* shampoos added with eucalyptus EO at 0.05 and 0.10 mL/cm^2 concentrations were more toxic to nymphs and adults of head lice than permethrin pediculicide (positive control; 68.0%-80.0% mortality rate for nymphs and 28.0%-36.0% mortality rate for adults). On the other hand, permethrin pediculicide was more toxic to adults of head lice than all formulations of *M. oleifera* shampoos. All formulations of *C. formosum* and *S. trilobatum* shampoos at high concentration (0.20 mL/cm^2) added with eucalyptus EO showed 12.0%-24.0% mortality rate of the eggs while permethrin pediculicide and all formulations of *M. oleifera* and *S. trilobatum* shampoos added with citrus EO did not show any ovicidal activity at all. Permethrin pediculicide was one of the most common pediculicides in Thailand for treatment of human pediculosis, but head lice resistance to it has caused treatment failure[6-8]. Similarly, many papers reported that permethrin pediculicide showed 20%-30% mortality rate against nymphs and adults of head lice but did not kill eggs[6,7,53,54]. Therefore, in order for a permethrin treatment to be effective, it needs to be applied repeatedly 2-3 times per week and treated for 7-10 d after the 1st application[7,54]. Unfortunately, permethrin pediculicide is persistent, harmful and highly toxic to human beings because it is neurotoxic and head lice resistance to it can develop. Common adverse effects after treatment with permethrin pediculicide are pruritus, burning and erythema[5-8,54]. For this reason, natural pediculicides derived from plant extracts are a more promising agent for head lice treatment. Our results showed that *C. formosum* + eucalyptus EO shampoo was highly effective pediculicide for killing two stage of head lice (nymphs and adults). The *C. formosum* + eucalyptus EO shampoo should not have any adverse effects to human beings since *C. formosum* has been edible for native Thai people for a long time and commonly used as traditional Thai herbal medicine for diarrhea, food poisoning, wound healing, cough, fever, and stomach ache[36,37]. The *C. formosum* + eucalyptus EO shampoo has a high potential for developing into new pediculicide for head lice treatment especially for infested Thai children whose head lice infestation rate was over 20%[55]. *C. formosum* + eucalyptus EO shampoo is inexpensive and safe. However, it should be officially tested for acute and chronic toxicity before it is used as herbal pediculicide for head lice treatment.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand for academic year 2015/2016, Grant No. KMITL 2560-01-04-003, and the National Research Council of Thailand (NRCT) (Thesis Grant for Doctoral Degree Student Fly 2016). We wish to thank the botanical taxonomist and entomologist at KMITL for their identification of plants and insects. We also wish to express our gratitude to Mr. Pratana Kangsadal, the KMITL Proofreader, for reviewing and giving comments on the manuscript.

References

[1] Sangare AK, Doumbo OK, Raoult D. Management and treatment of human lice. *Bio Med Res Inter* 2016; **2016**(2): 1-12.

[2] Meister L, Ochsendorf F. Head lice. *Dtsch Arztebl Int* 2016; **113**: 763-772.

[3] Walker C, Sebastian R, Krishna S, Tobias JD. A lousy reason for surgery cancellations. *Clin Pediatr (Phila)* 2016; **55**(8): 707-711.

[4] Durand R, Bouvresse S, Berdjane Z, Izri A, Chosidow O, Clark JM. Insecticide resistance in head lice: clinical, parasitological and genetic aspects. *Clin Microbiol Infect* 2012; **18**(4): 338-344.

[5] Feldmeler H. Pediculosis capitis: new insights into epidemiology,

diagnosis and treatment. *Eur J Clin Microbiol Infect Dis* 2012; **31**(9): 2105-2115.

[6] Wadowski L, Balasuriya L, Price HN, O'Haver J. Lice update: new solutions to an old problem. *Clin Dermatol* 2015; **33**(3): 347-354.

[7] Devore CD, Schutze GE. Head lice. *Pediatrics* 2015; **135**: e1355-1365.

[8] Clark JM, Yoon KS, Lee SH, Pittendrigh BR. Human lice: past, present and future control. *Pest Biochem Physiol* 2013; **106**(3): 162-171.

[9] Abdel-Ghaffar F, Abdel-Aty M, Rizk I, Al-Quraishy S, Semmler M, Gestmann F, et al. Head lice in progress: what could/should be done-a report on an *in vivo* and *in vitro* field study. *Parasitol Res* 2016; **115**(11): 4245-4249.

[10]Watcharawit R, Soonwera M. Pediculicidal effect of herbal shampoo against *Pediculus humanus capitis in vitro*. *Trop Biomed* 2013; **30**(2): 315-324.

[11]Soonwera M. Pediculicidal activities of herbal shampoo from *Zingiber officinale* Roscoe and *Camellia sinensis* (L.) Kuntze against head louse (*Pediculus humanus capitis* De Geer: Phthiraptera). *J Agri Tech* 2015; **11**(7): 1493-1502.

[12]Soonwera M. Toxicity of five herbal extracts against head louse (*Pediculus humanus capits* De Geer: Phthiraptera). *J Agri Tech* 2016; **12**(4): 657-666.

[13]Soonwera M. Efficacy of herbal shampoo base on native plant against head lice (*Pediculus humanus capitis* De Geer; Pediculidae: Phthiraptera) *in vitro* and *in vivo* in Thailand. *Parasitol Res* 2014; **113**(9): 3241-3250.

[14]Soonwera M. Herbal pediculicides base on *Alpinia galangal* (L.) Willd (Zingiberaceae) and *Syzygium aromaticum* (L.) Merrill& Perry (Myrtaceae) against head louse (*Pediculus humanus capitis* De Geer; Pediculidae). *J Agri Tech* 2015; **11**(7): 1503-1513.

[15]Bagavan A, Rahuman AA, Kamaraj C, Elango G, Zahir AA, Jayaseelan C, et al. Contact and fumigant toxicity of hexane flower bud extract of *Syzygium aromaticum* and its compounds against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Parasitol Res* 2011; **109**(5): 1329-1340.

[16]El-Wakeil NE. Botanical pesticides and their mode of action. *Gesunde Pflanzen* 2013; **65**(4): 125-149.

[17]George DR, Finn RD, Graham KM, Sparagano OA. Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. *Parasit Vectors* 2014; **7**(1): 1-12.

[18]Rahman JU, Ali A, Khan IA. Plant base products: use and development as repellents against mosquitoes: A review. *Fitoterapia* 2014; **95**(10): 65-74.

[19]Ebadollahi A. Iranian plant essential oils as sources of natural insecticide agents. *Int J Biol Chem* 2011; **5**(5): 266-290.

[20]Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: low-risk products in a high-stakes world. *Annu Rev Entomol* 2012; **57**(57): 405-424.

[21]Agra-Neto AC, Napoleão TH, Pontual EV, Santos ND, Luz Lde A, de Oliveira CM, et al. Effect of *Moringa oleifera* lectins on survivals enzyme activities of *Aedes aegypti* larvae susceptible and resistant to organophosphate. *Parasitol Res* 2014; **113**(1): 175-184.

[22]Yingngam B, Supaka N, Rungseevijitprapa W. Optimization of process parameters for phenolics extraction of *Cratoxylum formosum* ssp. *formosum* leaves by response surface methodology. *J Food Sci Technol* 2015; **52**(1): 129-140.

[23]Yingngam B, Monschein M, Brantner A. Ultrasound-assisted extraction of phenolic compounds from *Cratoxylum formosum* ssp. *formosum* leaves using central composite design and evaluation of its protective ability against H_2O_2-induced cell death. *Asian Pac J Trop Med* 2014; **7S1**: S497-505.

[24]Sahu J, Rathi B, Koul S, Khosa RL. *Solanum trilobatum* (Solanaceae)-an overview. *J Nat Remed* 2013; **13**(2): 76-80.

[25]Barbosa LC, Filomeno CA, Teixeira RR. Chemical variability and biological activities of *Eucalyptus* spp. essential oils. *Molecules* 2016; **21**(12): 1-33.

[26]Yones DA, Bakir HY, Bayoumi SAL. Chemical composition and efficacy of some selected plant oils against *Pediculus humanus capitis in vitro*. *Parasitol Res* 2016; **115**(8): 3209-3218.

[27]Gutierrez MM, Werdin-Gonzalez JO, Stefanazzi N, Bras C, Ferroro AA. The potential application of plant essential oils to control *Pediculus humanus capitis* (Anoplura: Pediculidae). *Parasitol Res* 2016; **115**(2): 633-641.

[28]Toloza A, Lucia A, Zerba E, Masuh H, Picollo MI. Interspecific hybridization of Eucalyptus as a potential tool to improve the bioactivity of Eos against permethrin-resistant head lice from Argentina. *Bioresour Technol* 2008; **99**(15): 7341-7347.

[29]Greive KA, Barnes TM. The efficacy of Australian essential oils for the treatment of head lice infestation in children: a randomized controlled trial. *Australas J Dermatol* 2017. Doi: 10.1111/ajd.12626.

[30]Toloza AC, Lucia A, Zerba E, Masuh H, Picollo MI. Eucalyptus essential oil toxicity against permethrin-resistant *Pediculus humanus capitis* (Phthiraptera: Pediculidae). *Parasitol Res* 2010; **106**(2): 449-414.

[31]Mehlhorn H, Abdel-Ghaffar F, Al-Rasheid KAS, Schmidt J, Semmler M. Ovicidal effects of neem seed extract preparation on eggs of body and head lice. *Parasitol Res* 2011; **109**(5): 1299-1302.

[32]Al-Quraishy S, Abdel-Ghaffar F, Mehlhorn H. Head louse control by suffocation to due to blocking their oxygen uptake. *Parasitol Res* 2015; **114**(8): 3105-3110.

[33]Dechakhamphu A, Wongchum N. Screening for anti-pancreatic lipase properties of 28 traditional Thai medicinal herbs. *Asian Pac J Trop Biomed* 2015; **5**(12): 1042-1045.

[34]Laphookhieo S, Maneerat W, Koysomboon S. Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Cratoxylum cochinchinense*. *Molecules* 2009; **14**(4): 1389-1395.

[35]Kuvatanasuchati J, Laphookhieo S, Rodanant P. Antimicrobial activity against periodontopathic bacteria and cytotoxic study of *Cratoxylum formosum* and *Clausena lansium*. *J Med Plants Res* 2011; **5**(25): 5988-5992.

[36]Ragasa C, Ng VAS, Shen CC. Chemical constituents of *Moringa oleifera* Lam. seeds. *Int J Pharm Phytochem Res* 2016; **8**(3): 495-498.

[37]Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales PN, Phivthong-ngam L, et al. The *in vitro* and *ex vivo* antioxidant properties, hydolipidaemic and antiatherosclerotic activities of water extract of

Moringa oleifera Lam. leaves. *J Ethnopharmacol* 2008; **116**(3): 439-446.

[38]Amabye TG. Chemical compositions and nutritional value of *Moringa oleifera* available in the market of Mekelle. *J Food Nutr Sci* 2015; **3**(5): 187-190.

[39]Thongpraditchote S, Hanchanga W, Wongkrajang Y, Temsiririrkkul R, Atisuk K. Toxicological evaluation of *Solanum trilobatum* L. fruits. *Mahidol U J Pharma Sci* 2014; **41**(4): 39-46.

[40]Ghaffar A, Yameen M, Kiran S, Kamal S, Jalal F, Munir B, et al. Chemical composition and *in-vitro* evaluation of the antimicrobial and antioxidant activities of essential oils extracted from seven *Eucalyptus* species. *Molecules* 2015; **20**(11): 20487-20498.

[41]Russo S, Cabrera N, Chludil H, Yaber-Grass M, Leicach S. Insecticidal activity of young and mature leaves essential oil from *Eucalyptus globulus* Labill. against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). *Chilean J Agri Res* 2015; **75**(3): 375-379.

[42]Grewick BC, Spark TC. Natural products for pest control: An analysis of their role, value and future. *Pest Manag Sci* 2014; **70**(8): 1169-1185.

[43]Priestley C, Burgess I, Williamson E. Lethality of essential oil constituents towards the human louse, *Pediculus humanus*, and its eggs. *Fitoterapia* 2006; **77**(4): 303-309.

[44]Waikedre J, Dugay A, Barrachina I, Herrenknecht C, Cabalion P, Fournet A. Chemical composition and antimicrobial activity of the essential oils from new Caledonian *Citrus macroptera* and *Citrus hystrix*. *Chem Biodivers* 2010; **7**(4): 871-877.

[45]Verzerza A, Trozzi A, Dugo G , Di Bella G, Cotroneo A. Biological lemon and sweet orange essential oils composition. *Flav Fragran J* 2004; **19**(6): 544-548.

[46]Michaelakis A, Papachristos D, Kimbaris A, Koliopoulos G, Giatropoulos A, Polissiou MG. Citrus essential oils and four enantiomeric pinenes against *Culex pipiens* (Diptera: Culicidae) *Parasitol Res* 2009; **105**(3): 769-773.

[47]Ferreira PM, Carvalho AF, Farias DF, Cariolano NG, Melo VM, Queiroz MG, et al. Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. *An Acad Bras Cienc* 2009; **81**(2): 207-216.

[48]Santos NDL, Moura KS, Napoleao TH, Santos N, Coelho LCBB, Navarro F, et al. Oviposition-stimulant and ovicidal activities of *Moringa oleifera* lectin on *Aedes aegypti*. *PLOS One* 2012; **7**: e44840. Doi:10.1371/journal.pone.0044840.

[49]Ashfaq M, Ashfaq U. Evaluation of mosquitocidal activity of water extract of *Maringa oleifera* seeds against *Culex quinquefasciatus* (Diptera: Culicidae) in Pakistan. *Pak Ento* 2012; **34**: 21-26.

[50]Permalatha S, Elumalai K, Jeyasankar A. Mosquitocidal properties of *Solanum trilobatum* leaf extracts against three important human vector mosquitoes (Diptera: Culicidae). *Asian Pac J Trop Med* 2013; **6**(11): 854-858.

[51]Keowkase R, Weerapreeyakul N. *Cratoxylum formosum* extract protects against amyloid-beta toxicity in a *Caenorhabditis elegans* model of Alzheimer's disease. *Planta Med* 2016; **82**(6): 516-523.

[52]Suddhasthira T, Thaweboon S, Dendoung N, Thaweboon B, Dechkunakorn S. Antimicrobial activity of *Cratoxylum formosum* on *Streptococcus mutans*. *Southeast Asian J Trop Med Public Health* 2006; **37**(6): 1156-1159.

[53]Lebwohl M, Clark L, Levitt J. Therapy for head lice based on life cycle, resistance, and safety considerations. *Pediatrics* 2007; **119**(5): 965-974.

[54]Eisenhower C, Ferrington EA. Advancements in the treatment of head lice in pediatrics. *J Pedriatr Health Care* 2012; **26**(6): 451-461.

[55]Rassami W, Soonwera M. Epidimiology of pediculosis capitis among schoolchildren in the eastern area of Bangkok, Thailand. *Asian Pac J Trop Biomed* 2012; **2**(11): 901-904.