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

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Objective: To evaluate the efficacy of Thai herbal shampoos ([*Cratoxylum 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/cm²) 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 LC₅₀ values of 0.004 and 0.005 mL/cm², 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...
compounds and monoterpenes but not of their pediculicidal activity against head lice and many papers that reported about the Thai herbs presented in this activity of acetylcholinesterase enzyme in the nervous system of EOs of constituents of cough, and pyrexia and as expectorants. The major chemical Thai herbs investigated in this study are also used in traditional Thai no negative effects on non-target organisms easily biodegradable, inexpensive and environmental friendly, having against head lice have been investigated for nymphicidal and adulticidal activities of acetylcholinesterase on the nervous system of insects and caused its paralysis and death. Natural pediculicides from plants have been investigated for nymyphicidal 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 C. aurantiifolius and C. hystrix fruits exhibited highly effective pediculicidal activity against head lice[30]. Melhorn et al[26] and Al-Quraishi et al[27] found that neem shampoo was highly toxic against eggs and adults of head lice. However, published papers related to ovicidal, nymyphicidal and adulticidal activities of Crotonyl formosanum (C. formosanum), 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. formosanum, S. trilobatum and M. oleifera, EOs of E. globulus and Citrus sinensis (C. sinensis) were phenoilic 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. formosanum, 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. formosanum 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 (KMITIL). 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°C 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 KMITIL [1. C. formosanum + eucalyptus EO shampoo (10% ethanolic extracts of C. formosanum leaves + 10% E. globulus EO + water + emulsifier), 2. C. formosanum + citrus EO shampoo (10% ethanolic extracts of C. formosanum leaves + 10% C. sinensis EO + water + emulsifier), 3. M. oleifera + eucalyptus EO shampoo (10% ethanolic extracts of M. oleifera seeds + 10% E. globulus EO + water + emulsifier), 4. M. oleifera + citrus EO shampoo (10% ethanolic extracts of M. oleifera seeds + 10% C. sinensis EO + water + emulsifier), 5. S. trilobatum + eucalyptus EO shampoo (10% ethanolic extracts of S. trilobatum seeds + 10% E. globulus EO + water + emulsifier), 6. S. trilobatum + citrus EO shampoo (10% ethanolic extracts of S. trilobatum seeds + 10% C. sinensis EO + water + emulsifier)]. All plant shampoos were stored in the laboratory (25°C 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.kmit.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 KMUTL 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 cmx7 cmx3 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/cm² 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 °C 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:

\[
\text{Mortality rate (\%) = } \frac{(NT/NC) \times 100}{100}
\]

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/cm²) with eucalyptus and citrus EOs supplements in terms of mortality rate and LC₅₀ 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/cm²) effected high mortality rates. The 0.20 mL/cm² of shampoo 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/cm² 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 pediculicide 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 LC₅₀ values of 0.004-0.300 mL/cm², while those added with 10% citrus EO showed LC₅₀ values of 0.005-0.400 mL/cm². The LC₅₀ 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/cm², respectively, and the LC₅₀ value of C. formosum shampoo added with 10% citrus EO was 43.8 mL/cm². The LC₅₀ values of permethrin pediculicide against nymphs and adults were 0.020 and 0.040 mL/cm², respectively. Drinking water did not show any LC₅₀ value. The relationships between mortality rate (%) and concentrations (0.05, 0.10 and 0.20 mL/cm²) 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/cm², the mortality rate of nymphs and adults of head lice increased. The high concentration (0.2 mL/cm²) 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.
Herbal shampoos added with 10% eucalyptus EO at 0.05 mL/cm² (control) and drinking water (negative control) were significantly higher than permethrin pediculicide (positive control). The nymphicidal and adulticidal activities of all formulation of Thai herbal shampoos and permethrin pediculicide and drinking water at 7 d against the eggs and at 5 min against nymphs and adults of head lice was 0.20 mL/cm² 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-pinene, β-pinene, 2-β-pinene, β-myrcene, α-phellandrene, 1-isopropenyl-3-methylbenzene, γ-terpinene, (E)-pinocarveol and 1-α-terpineol. 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 eggs and adults and inhibited their tracheal 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.

### 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).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mL/cm²)</th>
<th>Egg Mortality (%)</th>
<th>Nymph Mortality (%)</th>
<th>Adult Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. formosum + eucalyptus EO shampoo</td>
<td>0.05</td>
<td>12.0±9.4</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>16.0±6.9</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>24.0±5.7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C. formosum + citrus EO shampoo</td>
<td>0.05</td>
<td>0</td>
<td>88.0±8.9</td>
<td>76.0±8.9</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0</td>
<td>96.0±8.9</td>
<td>88.0±10.9</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>12.0±10.9</td>
<td>100</td>
<td>96.0±8.9</td>
</tr>
<tr>
<td>M. oleifera + eucalyptus EO shampoo</td>
<td>0.05</td>
<td>4.0±3.9</td>
<td>76.0±8.9</td>
<td>20.0±5.2</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>8.0±5.5</td>
<td>92.0±9.5</td>
<td>28.0±12.8</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>12.0±7.5</td>
<td>100</td>
<td>32.0±8.8</td>
</tr>
<tr>
<td>M. oleifera + citrus EO shampoo</td>
<td>0.05</td>
<td>0</td>
<td>68.0±10.9</td>
<td>20.0±8.9</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0</td>
<td>88.0±14.1</td>
<td>22.0±9.5</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>96.0±8.9</td>
<td>30.0±7.5</td>
<td></td>
</tr>
<tr>
<td>S. trilobatum + eucalyptus EO shampoo</td>
<td>0.05</td>
<td>4.0±2.2</td>
<td>92.0±8.3</td>
<td>84.0±6.8</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>12.0±5.5</td>
<td>92.0±8.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>12.0±4.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>S. trilobatum + citrus EO shampoo</td>
<td>0.05</td>
<td>0</td>
<td>84.0±10.9</td>
<td>80.0±7.8</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0</td>
<td>88.0±8.9</td>
<td>86.0±8.9</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>98.0±7.9</td>
<td>96.0±7.8</td>
<td></td>
</tr>
<tr>
<td>Permethrin pediculicide (positive control)</td>
<td>0.05</td>
<td>0</td>
<td>68.0±9.8</td>
<td>28.0±7.8</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0</td>
<td>80.0±11.3</td>
<td>36.0±6.7</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>92.0±8.5</td>
<td>60.0±5.8</td>
<td></td>
</tr>
<tr>
<td>Drinking water (negative control)</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

### Table 2

LC₅₀ 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/cm²).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Egg</th>
<th>Nymph</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. formosum + eucalyptus EO shampoo</td>
<td>33.4</td>
<td>0.004</td>
<td>0.005</td>
</tr>
<tr>
<td>C. formosum + citrus EO shampoo</td>
<td>43.8</td>
<td>0.005</td>
<td>0.006</td>
</tr>
<tr>
<td>M. oleifera + eucalyptus EO shampoo</td>
<td>44.6</td>
<td>0.010</td>
<td>0.300</td>
</tr>
<tr>
<td>M. oleifera + citrus EO shampoo</td>
<td>NA</td>
<td>0.020</td>
<td>0.400</td>
</tr>
<tr>
<td>S. trilobatum + eucalyptus EO shampoo</td>
<td>44.8</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td>S. trilobatum + citrus EO shampoo</td>
<td>NA</td>
<td>0.006</td>
<td>0.007</td>
</tr>
<tr>
<td>Permethrin pediculicide (positive control)</td>
<td>NA</td>
<td>0.020</td>
<td>0.040</td>
</tr>
<tr>
<td>Drinking water (negative control)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

LC₅₀ 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/cm² 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/cm² concentration were more highly toxic to nymphs and adults of head lice than those added with 10% citrus EO. The maximum
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 activities 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 quinquefasciatus 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/cm² 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 added with eucalyptus EO at 0.20 mL/cm²) 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.

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