
Herbal Pediculicides Base on *Alpinia galanga* (L.) Willd (Zingiberaceae) and *Syzygium aromaticum* (L.) Merrill and Perry (Myrtaceae) against Head Louse (*Pediculus humanus Capitis* De Geer; Pediculidae)

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Abstract Currently, head lice infestations are one of the most common human infections in the world, especially in kindergarten and in primary schoolchildren aged between 3 and 15 years. Moreover, head lice resistance to chemical insecticides have increased. However, new alternative topical therapies for head lice infestations are needed, especially those containing plant-derivative active ingredients. Therefore, the present study, we reported the efficacy of two herbal shampoos based on *Alpinia galanga* (L.) Willd and *Syzygium aromaticum* (L.) Merrill & Perry against head lice and compare them with malathion shampoo (A-Lice shampoo®: 1% w/v malathion) and baby shampoo (Babi Mild Natural' N Mild®) in order to assess their in vitro efficacy. The results revealed that all herbal shampoo at 6 µl/cm² was more effective pediculicide than at 3 µl/cm², malathion shampoo and baby shampoo. The highest pediculicidal activity was shown by *A. galanga* shampoo at dose of 6 µl/cm² with 100% mortality at 3 min, LT₅₀ value of 0.4 min and LC₅₀ value of 1.8 µl/cm². However, 6 µl/cm² of *S. aromaticum* shampoo showed 100% mortality at 10 min, LT₅₀ value of 0.9 min and LC₅₀ value of 2.3 µl/cm². While, malathion shampoo showed low toxicity with 34.5% mortality at 10 min and LT₅₀ value of 35.9 min and baby shampoo showed non-toxic to all head lice during the observation periods. All data in this study showed that two herbal shampoos base on *A. galanga* and *S. aromaticum* have high potential of alternative pediculicides for head lice treatments of children in Thailand.

Keywords: *Pediculus humanus capitis*, herbal pediculicide, *Alpinia galanga*, *Syzygium aromaticum*

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Introduction

Infestation with head lice (*Pediculus humanus capitis* : Phthiraptera ;Pediculicidae) is a worldwide problem, mainly affecting kindergarten and children aged between 3 and 15 years (Mehlhon, 2012), in all socioeconomic back grounds(Greive and Barnes, 2012) or in both developed and underdeveloped nations (Gallardo *et al.*, 2009). Each year, million of children are infested with head lice , a condition known as pediculosis capitis , which is responsible for tens of million of lost school days (Bagavan *et al.*, 2011). However, the number of head lice infestation case has increased world wide since the mid-1960S, reaching hundreds of millions annually (Falagas *et al.*,2008). Moreover, an increased rate of head lice infestations were reported in recent years from a number of countries including North and South America, Europe, Asia and Australia (Burkhart and Burkhart, 2002; Bagavan *et al.*, 2011). The high levels of infestations have also been reported from all over the world, ranging from 1.8 to 87.0% (Falagas *et al.*, 2008). However, 4-8 million of children (3-12 years of age) in USA are treated unnecessarily for head lice annually and 12-24 million school days are lost annually. Moreover, annual economic loss owing to missed workdays by parent who have to stay home with their children adds US\$ 4-8 billion to country's economy (Bagavan *et al.*, 2011; Mumcuoglu *et al.*, 2006). In Thailand, head lice infestation is a serious problem affecting more than 20% of the primary schoolchildren in the eastern area of Bangkok and more than 80% of the primary schoolchildren in Ratchaburi Province, this area is near the Thai-Myanmar border(Rassami and Soonwera, 2012; Thanyavanich *et al.*, 2009). In general, adult of head lice that successfully feed on a scalp of human may survive up to 30 days but cannot live more than 48 hours away from a feeding source (Eisenhower and Farrington, 2012). In addition, both saliva and fecal matter from head lice may lead to inflammation and itching of the human's scalp and chronic heavy case may lead to anaemia and head lice infestation cause not only physical symptoms but also psychological distress , since infested children believe that head lice infestation is a result of being dirty ,and social blame (Oh *et al.*, 2010; Mumcuoglu *et al.*, 2006; Rassami and Soonwera, 2013a; Soonwera, 2014; Speare *et al.*, 2006). However, head lice are only known to crawl, not jump, hop or fly (they are wigless), so they can be transmitted through close personal contact or sharing of personal items such as hairbrushes, hats, pillows or other personal items of infected person. Thus, transmission in most cases occurs by personal contact with head-to-head (Burkhart and Burkhart, 2007; Frankowski and Bocchini, 2010). Currently, chemical control of head lice has been performed using a wide variety of chemical insecticides from DDT, lindane,

malathion and synthetic pyrethroids (permethrin, phenotrin). In addition, resistance of head lice to permethrin, phenotrin, lindane, malathion have increased and reported in several countries (in France, UK, Australia and Denmark) (Burkhart and Burkhart, 2000; Eisenhower and Farrington, 2012; Durand *et al.*, 2012). Moreover, many chemical pediculicides may harm health of children or may produce residuals inside the children's body. Some researcher reported that, malathion insecticide has been found to disrupt the immune system and carbaryl insecticide is a potential human carcinogen (any substance that produce cancer) (Abdel-Ghaffar and Semmler, 2007). Consequently, in recent times, many natural products from plants, herbs have been suggested as an alternative source of materials for head lice treatment because they constitute a rich source of bioactive chemicals and are commonly used as flavoring agents for foods and beverages and low mammalian toxicity (Marimuthu *et al.*, 2012). Several plant products, such as aniseed, clove, coconut, neem, tea tree oils, henna and long pepper were used in different available compositions for the treatment of head lice infestations (Bagavan *et al.*, 2011; Bankar *et al.*, 2011; Rassami and Soonwera, 2011). Thus, we carried out this study in order to evaluate the susceptibility of head lice to herbal shampoos from *Alpinia galanga* and *Syzygium aromaticum* and to compare the efficacy of two herbal shampoos with malathion shampoo and baby shampoo in vitro. However, *Syzygium aromaticum*, commonly known as clove, belonging to family Myrtaceae, it is an evergreen tree, native of Indonesia and Mallaca island (Alma *et al.*, 2007). The essential oils from clove showed mainly contained about eugeno (Chudiwal *et al.*, 2010; Shapiro, 2012) and clove oil is commonly used in dental root canal surgery for its antimicrobial properties (Chudiwal *et al.*, 2010). *Alpinia galanga*, belonging to family Zingiberaceae, commonly known as galangal. It is an important cultivated medicinal plant of India and Asia. It is well known official drug throughout the country as a holistic gift of nature for medicinal, culinary and cosmetic use, and it has been found to possess various therapeutic activities such as anti-inflammatory, analgesic, antiallergic, antifungal, antibacterial, antidiabetic, antiulcer and many more (Chudiwal *et al.*, 2010).

Materials and methods

Plant materials

The rhizomes of *A. galanga* and flower buds of *S. aromaticum* were collected from Phangchong district, Nakhornratchasima province, the North-Eastern of Thailand, during January to February 2014. The taxomic

identification was made by plant taxonomist from Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. The voucher specimens were numbered and kept in laboratory of medicinal plants, KMITL, for further reference.

Herbal shampoo

Two herbal shampoos base on *A. galanga* and *S. aromaticum* were formulated and provided by the medicinal plant scientist, Medicinal Plant Laboratory, Faculty of Agricultural Technology, KMITL.

Chemical pediculicide (positive control) and baby shampoo (negative control)

Chemical pediculicide

Malathion shampoo (A-Lice shampoo[®]; 1.0% w/v malathion) is a common chemical pediculicide in Thailand, was purchased from HOE Pharmaceuticals Shn. Bhd. Lot 10, Jalan Sultan Mohd.6, Bandar Sultan Suleiman, 42000 Port Klang, Malaysia and used as positive control.

Baby shampoo

Babi Mild Natural' N Mild[®] is a common shampoo for Thai children, it was purchased from Greensville Co. Ltd. Chalongkrug 31, Lumplatiue, Ladkrabang, Bangkok, Thailand and used as negative control.

Collection of head lice

The protocol for head lice collection was approved by the director of 3 primary schools located in Ladkrabang area, Bangkok, Thailand and in collaboration with primary school teachers and parents of primary school children. The schoolchildren had not been treated with any chemical or herbal pediculicides for at least the preceding month and allowed using only the fire-toothed comb. Collection of head lice were collected from a population of schoolchildren between the ages of 5 to 10 years by raking a plastic fire-toothed comb through sections of the children's scalp. Head lice were obtained and pooled by carefully removing them from teeth of the comb into clean insect boxes (18x23x5.5 cm), during May to June, 2014. Then, head lice were transported to Laboratory of Entomological, KMITL. The head lice were

identified by Entomologist from Faculty of Agricultural Technology, KMITL. The head lice specimens were kept in Entomological Museum, KMITL, for further reference.

Filter paper contact toxicity bioassay

The Entomological laboratory, KMITL far from three primary schools about 15 min, so head lice were transported to Entomological laboratory within 30 min and in vitro tests were started, using filter paper contact toxicity bioassay (Rassami and Soonwera, 2013a; Soonwera, 2014), at doses of 3 and 6 $\mu\text{l}/\text{cm}^2$. Each dose of herbal shampoo was applied to the filter paper (Whatman[®] No 1; 4.5 cm in diameter). After drying for 30 s, each filter paper was placed on the bottom of a petri dish (5.0 cm in diameter). The groups of ten head lice adults were placed on each petri dish. Malathion shampoo (A-Lice shampoo[®]) run as positive control and baby shampoo (Babi Mild Natural' N Mild[®]) run as negative control were simultaneously. Treated and control head lice were held at the same condition (32.5 ± 1.2 °C and 64.8 ± 3.5 % relative humidity (RH)). Adult mortalities were recorded at 1, 5, and 10 min, under stereomicroscope. The mortality criteria of head lice were according to Rassami and Soonwera (2013b) and Soonwera (2014). All treatments were replicated 10 times. The Median Lethal Time (LT₅₀) and Median Lethal Concentration (LC₅₀) values were calculated by probit analysis (SPSS for Windows version 16.0). Moreover, adult mortality data was analyzed with Duncan's Multiple Range Test (DMRT).

Results

The result in Table1, 2 and 3

The pediculicidal activity of *A. galanga* shampoo, *S. aromaticum* shampoo at doses of 3 and 6 $\mu\text{l}/\text{cm}^2$ against head lice (*P. humanus capitis*) and compare them with malathion shampoo (A-Lice shampoo[®]: 1% w/v malathion) and baby shampoo (Babi Mild Natural' N Mild[®]), are shown in Table 1 and 2, respectively. However, LT₅₀ values in minutes and LC₅₀ values in $\mu\text{l}/\text{cm}^2$ of two herbal shampoos, malathion shampoo and baby shampoo against head lice is shown in Table 3. All head lice treated with all herbal shampoo at doses of 3 $\mu\text{l}/\text{cm}^2$ showed 40.6-74.4, 53.2-83.8 and 65.2-92.2% mortality at 1, 5 and 10 min, respectively and LT₅₀ values ranged from 0.8-5.4 min. Meanwhile, 100% of head lice in negative control (baby shampoo) survived during the experiment periods. Moreover, malathion shampoo caused 12.4 ± 8.7 % mortality at 10 min.

The most effective pediculicide was *A. galanga* shampoo with 92.2±4.8% mortality at 10 min and LT₅₀ values of 0.8 min. For dose of 6 µl/cm², *A. galanga* shampoo also exhibited the most effective pediculicide with 100% mortality at 5 min, LT₅₀ values of 0.4 min and LC₅₀ values of 1.8 µl/cm², followed by *S. aromaticum* shampoo and malathion shampoo with 77.8±10.5 and 20.0±7.8% mortality and LT₅₀ values of 0.9 and 35.9 min and LC₅₀ values of 2.3 and 19.8 µl/cm², respectively. On the other side, baby shampoo showed non toxicity to head lice, so all head lice treated with baby shampoo survived during experiment periods. However, 6 µl/cm² of *S. aromaticum* shampoo caused 100% mortality of head lice at 10 min. While, malathion shampoo showed 34.5±10.5% mortality at 10 min. On the median lethal concentrations (LC₅₀) values indicated the order of pediculicidal activity in the treatment as *A. galanga* shampoo > *S. aromaticum* shampoo > malathion shampoo > baby shampoo.

Table 1. Toxicity of herbal shampoos, chemical shampoo and baby shampoo on mortality of human head lice at 3 µl/cm².

Treatment	% Mortality ±SD/time (min)		
	1.0	5.0	10.0
<i>A. galanga</i> shampoo	74.4±5.2a ^{1/}	83.8±3.9a	92.2±4.8a
<i>S. aromaticum</i> shampoo	40.6±10.7b	53.2±9.9b	65.2±7.3b
malathion shampoo (A-Lice shampoo [®])	0c	0c	12.4±8.7c
baby shampoo (Babi Mild Natural' N Mild [®])	0c	0c	0d

^{1/} Percent mortality 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. Toxicity of herbal shampoos, chemical shampoo and baby shampoo on mortality of human head lice at 6 $\mu\text{l}/\text{cm}^2$.

Treatment	% Mortality \pm SD/time (min)		
	1.0	5.0	10.0
<i>A. galanga</i> shampoo	92.6 \pm 4.0a ^{1/}	100a	100a
<i>S. aromaticum</i> shampoo	51.2 \pm 5.0b	77.8 \pm 10.5b	100b
malathion shampoo (A-Lice shampoo [®])	0c	20.0 \pm 7.8c	34.5 \pm 10.4b
baby shampoo (Babi Mild Natural' N Mild [®])	0c	0d	0c

^{1/} Percent mortality 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 3. LT₅₀ values in minutes and LC₅₀ values in $\mu\text{l}/\text{cm}^2$ of herbal shampoos, malathion shampoo and baby shampoo against head lice (*P. humanus capitis*).

Treatment	LC ₅₀ ^{1/} values in $\mu\text{l}/\text{cm}^2$ at 5 min	LT ₅₀ ^{2/} values at 3 $\mu\text{l}/\text{cm}^2$	LT ₅₀ values at 6 $\mu\text{l}/\text{cm}^2$
<i>A. galanga</i> shampoo	1.8	0.8	0.4
<i>S. aromaticum</i> shampoo	2.3	5.4	0.9
malathion shampoo (A-Lice shampoo [®])	19.8	ns ^{3/}	35.9
baby shampoo (Babi Mild Natural' N Mild [®])	ns	ns	ns

^{1/} LC₅₀ values = median lethal concentration, ^{2/} LT₅₀ values = median lethal time

^{3/} ns = not computed by Probit analysis

Discussion

The most effective pediculicide was shown by *A. galanga* shampoo with 100% mortality at 5 min, LT₅₀ values ranged from 0.4-0.8 min and LC₅₀ value of 1.8 $\mu\text{l}/\text{cm}^2$, followed by *S. aromaticum* shampoo with 100% mortality at 10 min, LT₅₀ values ranged from 0.9-5.4 min and LC₅₀ value of 2.3 $\mu\text{l}/\text{cm}^2$. However, two herbal shampoo were more effective pediculicide than malathion shampoo (A-Lice shampoo[®]) and baby shampoo (Babi Mild Natural' N Mild[®]). Thus, baby shampoo is commonly shampoo for children but can not used as pediculicide, and Rassami and Soonwera (2013) reported that 100% of head lice in negative control group (baby shampoo : Babi Mild Natural' N Mild[®]). In

addition, *A. galanga* shampoo and *S. aromaticum* shampoo have been suggested as an new alternative products for head lice treatment, since two herbal shampoo are safe alternatives due to their non-toxicity to human, especially children than chemical shampoo. However, *A. galanga* commonly known as galangal belonging family Zingiberaceae, is an important cultivated medicinal crop of India and Asia including Thailand. It is well known official drug and it has been found to possess various therapeutic activities such as anti-inflammatory, analgesic, antiallergic, antifungal, antibacterial, antiulcer, immunostimulating, antidiabetic, anticancer, antioxidant, antiamebic, antidermatophytic and many more (Chudiwal *et al.*, 2010). Moreover, the main components of the essential oils of *A. galangal* were monoterpenes, monoterpene alcohols and ester, sesquiterpenes, methyleugenol, eugenol acetate, chavicol and chavicol acetate (Sukhirun *et al.*, 2011). In addition, many researchers reported that extracts of the rhizome of *A. galanga* exert significant larvicidal activities against *Plutella xylostella* (Lepidoptera) (Trakranrugsie *et al.*, 2008) and the rhizome of *A. galangal* are used as stomachic, carminative, antifatulent, antifungal, anti-itching, dog mange and weeping wound (Sukhirun *et al.*, 2011; Trakranrugsie *et al.*, 2008). However, *S. aromaticum*, commonly known as clove, is belonging family Myrtaceae, It is an evergreen tree, native of Indonesia and Malacca island distributed in tropic of the old world. Moreover, it is natural analgesics and antiseptic used primarily in dentistry for its main ingredient eugenol (Alma *et al.*, 2007; Chaieb *et al.*, 2007). Bagavan *et al.* (2011) reported that hexane flower bud extract of *S. aromaticum* showed pediculicidal activity against head lice. In addition, Soonwera (2015) reported that *S. aromaticum* essential oil exhibited larvicidal and oviposition deterrent activities against house fly (*Musca domestica*, Diptera) and these results were more effective than cypermethrin. On the other side, malathion shampoo showed 12.4±8.7 to 34.5±10.4% mortality, LT₅₀ values of 35.9 min and LC₅₀ values of 19.8 µl/cm², and these results was less pediculicidal activity than *A. galanga* shampoo and *S. aromaticum* shampoo. Moreover, malathion is a neurotoxic organophosphorus insecticide, that inhibits cholinesterase activity of head lice (Durand *et al.*, 2012 ; Eisenhower and Farrington, 2012). However, malathion was first approved for treatment of head lice 2 decades ago but was withdrawn from the us market by the manufacturer because of the better convenience and cosmetic appeal of over-the-counter products, but recently in the US, 0.5% malathion was reintroduced (Burkhart and Burkhart, 2002) . The use of malathion in patients younger than 6 years is not recommended, and its use is contraindicated for neonates and infants because of increased permeability of the scalp and malathion can be irritating to the scalp and skin, and ophthalmic exposure can lead to mild conjunctivitis (Eisenhower and Farrington, 2012) . Moreover, head lice resistance to

malathion has been reported in Europe, and first reported in France and then in the UK, Australia and Denmark (Durand *et al.*, 2012). The effectiveness of malathion in a single-blind, randomized study in the UK was poor, showing cure rates of only 17.0% (Durand *et al.*, 2012). Thus, using the herbal shampoos base on *A. galanga* and *S. aromaticum* for head lice treatments are and better and safe alternative products than to use the malathion shampoo or other chemical shampoos, and two herbal shampoos will be served as new alternative pediculicides for Thai children in the future.

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