
Pediculicidal Activities of Herbal Shampoos from *Zingiber officinale* Roscoe and *Camellia sinensis* (L.) Kuntze Against Head Louse (*Pediculus humanus capitis* De Geer:Phthiraptera)

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Soonwera, M. (2015). Pediculicidal activities of herbal shampoos from *Zingiber officinale* Roscoe and *Camellia sinensis* (L.) Kuntze against head louse (*Pediculus humanus capitis* De Geer:Phthiraptera). International Journal of Agricultural Technology 11(7):1519-1528.

Abstract The increasing resistance of head lice ((*Pediculus humanus capitis* De Geer:Phthiraptera:Pediculidae) to chemical pediculicides has been documented world wide, including Thailand. Moreover, chemical pediculicides have lost their efficacy and thus alternative products such as herbal essential oils, herbal shampoos have been proposed to treat head lice infestation. Therefore, the present study investigated the efficacy of two herbal shampoos based on ginger (*Zingiber officinale* Roscoe; Zingiberaceae and tea (*Camellia sinensis* (L.) Kuntze:Theaceae) against head lice and compared them with chemical pediculicide (positive control ; 1% w/w lindane, Hexin Lice Killer[®]) and baby shampoo (negative control ; Johnson's baby soft shiny shampoo[®]) in order to assess their in vitro efficacy. The results showed that all herbal shampoo was more effective than chemical shampoo and baby shampoo with 100% mortality at 10 min. and LT₅₀ values ranged from 0.4 to 1.4 min. and LC₅₀ values ranged from 1.6 to 3.2 µl/cm². Meanwhile, chemical pediculicide and baby shampoo showed non toxicity to head lice, so 100% of head lice in chemical and baby shampoos groups survived during the observation periods(10 min). Our data demonstrates the high potential of herbal shampoos from *Z. officinale* and *C. sinensis* to be used as pediculicides against head lice in Thailand

Keyword: Head louse, *Zingiber officinale*, *Camellia sinensis*, herbal shampoo

Introduction

Head lice infestation caused by *Pediculus humanus capitis* De Geer is an important public health problem throughout the world, especially in children aged 3 to 12 years in both developed and developing countries including Thailand (Bush *et al.*, 2011; Gallard *et al.*, 2009). Moreover, head lice feed on only human blood at the scalp and neck area and they cause itching and inflammation of the scalp and neck, occasional secondary bacterial infestation that may lead to anemia (Canadian Pediatric Society, 2008; Frankoski and Bocchini, 2010; Lapeere *et al.*,

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2014). However, the control of head lice worldwide depends on chemical insecticides such as lindane, malathion, carbaryl and permethrin, despite that chemical insecticides are very harmful for human health and very toxic for children. Moreover, the increasing resistance to chemical insecticides used for the control of head lice infestation has been documented over the last decade. Additionally, treatment failure and head lice tolerance to chemical insecticides have been reported in several countries such as Australia, Argentina, UK and USA (Burgess, 2008; Burgess and Burgess, 2011; Clark, 2009; Gur and Schneeweiss, 2009). However, many plant-based products have been suggested as alternative products for head lice control because they are good and safe alternative products due to their less toxicity to human than chemical insecticides and they are easy biodegradability (Bagaven *et al.*, 2011; Heukelbach *et al.*, 2006a; 2006b).

Thus, alternative pediculicides for head lice treatments are needed throughout the world, especially those containing plant-derivative active ingredients (Rassami and Soonwera, 2013a). In addition, many researchers reported that products based on plants or herbs showed the pediculicidal activities such as *Zingiber cassumunar*, *Piper betle*, *Piper ribesioides*, *Averrhoa bilimbi*, *Azadirachta indica*, *Eucalyptus sp*, *Accacia concinna*, *Tamarindus indica*, *Acorus calamus*, *Phyllanthus emblica* and *Zanthoxylum limonella* (Heukelbach *et al.*, 2008; Soonwera, 2014; Rassami and Soonwera, 2013b; Abdel-Ghaffar *et al.*, 2012). Therefore, the aim of this study was to evaluate the potential of pediculicidal activity of two herbal shampoos from rhizomes of ginger (*Zingiber officinale*) and leaves of tea (*Camellia sinensis*) against head lice and to compare them with chemical pediculicide (1% w/w lindane, Hexin Lice Killer[®]) as positive control and baby shampoo (Johnson's baby soft shiny shampoo[®]) as negative control. However, *Z. officinale* and *C. sinensis* are native plants of Asia including Thailand, belonging to the family Zingiberaceae and Theaceae, respectively. *Z. officinale* is a common condiment for various foods and beverages and has a long history of important traditional medicine herb for the treatment of stomach disorders. In addition, rhizome of *Z. officinale* has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds (Bhargava *et al.*, 2012). The anti-inflammatory and antioxidant and antimicrobial properties of *Z. officinale* have been known and valued for centuries and it is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse or side effects (Bhargava *et al.*, 2012; Hasan *et al.*, 2012; Sasidharan and Menon, 2010). *C. sinensis* is one of the most widely consumed beverages in the world and its medicinal properties have been widely explored (Namita *et al.*, 2012). However, *C.*

sinensis is a native to mainland China, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. The anti-aging, anti-Alzheimer, antiparkinson, antistroke, anticancer, antidiabetic and anticaries activities of *C. sinensis* have been known in several countries (Namita *et al.*, 2012; Sherwani *et al.*, 2013).

Materials and methods

Plant materials and herbal shampoos

The leaves of *C. sinensis* and rhizomes of *Z. officinale* were collected from the local market of Ladkrabang district, Bangkok, Thailand in January, 2015. The taxonomic identification of two plants in this study were made by plant taxonomist of Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The herbal shampoo were formulated by scientist of the Medicinal Plant Laboratory, Faculty of Agricultural Technology, KMITL.

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

a. Lindane 1% w/w (Hexin Lice Killer[®]) is a common chemical pediculicide in Thailand, it was purchased from Krungdheub Pharmacy LTD, PART. 783 Charoennakorn Rd, Bang-pakok, Rasburana, Bangkok 10140, Thailand and used as positive control.

b. Baby shampoo (Johnson's baby soft shiny shampoo[®]), is a common baby shampoo for children in Thailand, it was purchased from Johnson (Thai) Pte, LTD, 106 Moo 4, Chalongkrug Road, Ladkrabang, Bangkok 10520, Thailand used as negative control.

Collection of head lice (P. humanus capitis)

The protocol for head lice collection was approved by the director of three primary schools and in collaboration with primary school teachers and parents. *P. humanus capitis* were collected by fire-toothed combs from 120 infested primary schoolchildren between the age of 7-10 years at primary schools located in Ladkrabang district Bangkok, Thailand, during March, 2015 to May, 2015. After collection, head lice were transported to Entomological Laboratory, KMITL, head lice were identified by Entomologist of Faculty of Agricultural Technology, KMITL and head lice specimens were kept in Entomological laboratory, KMITL for further reference.

Bioassay

The bioassay was started within 30 min. after collection of head lice and using a filter paper contact (Soonwera,2014). Doses of 3 $\mu\text{l}/\text{cm}^2$ and 6 $\mu\text{l}/\text{cm}^2$ of each herbal shampoo were applied to the filter paper (Whatman[®] No. 1, 4.5cm. in diameter), and after during for 30s, each filter paper was placed on the bottom of a petri dish (5.0 cm. diameter). Afterward, selection of 10 head lice were placed on the filter paper. However, lindane and baby shampoo simultaneously run as positive and negative control, respectively. The mortality of head lice on the filter paper were recorded under stereomicroscope at 1, 5 and 10 min. The criteria for mortality of head lice were strict and were defined as the complete absence of any vital signs such as gut movement, movement of limbs, antennae or movement of legs with or without stimulation using forceps(Rassami and Soonera, 2013a). All treatments were replicated 10 times. The mortality data was analyzed with Duncan's multiple range test (DMRT) using SPSS for windows version 16.0 The LT_{50} value (median lethal time) and LC_{50} value (median lethal concentration) were calculated using probit analysis.

Results

The results of in vitro tests, the insecticidal activity of two herbal shampoos from *Z. officinale* and *C. sinensis* at 3 and 6 $\mu\text{l}/\text{cm}^2$ against head lice and were compared with chemical pediculicide (Lindane: Hexin Lice Killer[®]) and Baby shampoo (Johnson's baby soft shiny shampoo[®]) as shown in Table 2 and 3, respectively. All head lice treated with herbal shampoos showed 100% mortality at 10 min., LT_{50} values ranged from 0.4 to 1.4 min and LC_{50} values ranged from 1.6 to 3.2 $\mu\text{l}/\text{cm}^2$ (Table 3). On the other side, 100% of head lice in chemical pediculicide (positive control) and baby shampoo (negative control) survived during observation periods (10 min). The most effective pediculicide was *Z. officinale* shampoo with 100% mortality at 5 min and LT_{50} values ranged from 0.4 to 0.07 min and LC_{50} values ranged from 1.6 $\mu\text{l}/\text{cm}^2$. In addition, this results showed significant pediculicidal activity (LT_{50} values $< 1.0 \text{ min}^{[17]}$). For 3 and 6 $\mu\text{l}/\text{cm}^2$ *C. sinensis* shampoo showed 78.4 and 100% mortality at 10 min and LT_{50} values ranged from 1.2 to 1.4 min, LC_{50} value was 3.2 $\mu\text{l}/\text{cm}^2$. Moreover, all herbal shampoo at 6 $\mu\text{l}/\text{cm}^2$ was more effective pediculicide than 3 $\mu\text{l}/\text{cm}^2$. In addition, all herbal shampoo at all concentration also showed significant differences over chemical pediculicide and baby shampoo ($p < 0.05$).

Table 1. LT₅₀ values (min) and LC₅₀ values (µl/cm²) against human head lice among two herbal shampoos, chemical pediculicide and baby shampoo.

Treatment	LT ₅₀ ^{1/} value (min) at 3 µl/cm ²	LT ₅₀ value (min) at 6 µl/cm ²	LC ₅₀ ^{2/} value µl/cm ² at min
<i>Z. officinale</i> shampoo	0.7	0.4	1.6
<i>C. sinensis</i> shampoo	1.4	1.2	3.2
Chemical pediculicide (lindane)	ns	ns	ns
Baby shampoo (Johnson's baby soft shiny shampoo [®])	ns	ns	ns

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

ns = not computed by Probit analysis

Table 2. Effect of two herbal shampoos, chemical pediculicide and baby shampoo on mortality of head lice at 3 µl/cm²

Treatment	Mortality ±SD/time (min)		
	1	5	10
<i>Z. officinale</i> shampoo	86.0±3.5a ^{1/}	100a	100a
<i>C. sinensis</i> shampoo	55.2±5.4b	61.2±5.3b	78.4±2.7b
Chemical pediculicide (lindane)	0c	0c	0c
Baby shampoo (Johnson's baby soft shiny shampoo [®])	0c	0c	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. Effect of two herbal shampoos, chemical pediculicide and baby shampoo on mortality of head lice at 6 $\mu\text{l}/\text{cm}^2$

Treatment	Mortality \pm SD/time (min)		
	1	5	10
<i>Z. officinale</i> shampoo	92.2 \pm 3.5a ^{1/}	100a	100a
<i>C. sinensis</i> shampoo	56.4 \pm 5.2b	72.6 \pm 4.0b	100a
Chemical pediculicide (lindane)	0c	0c	0b
Baby shampoo (Johnson's baby soft shiny shampoo [®])	0c	0c	0b

^{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, $p < 0.05$)

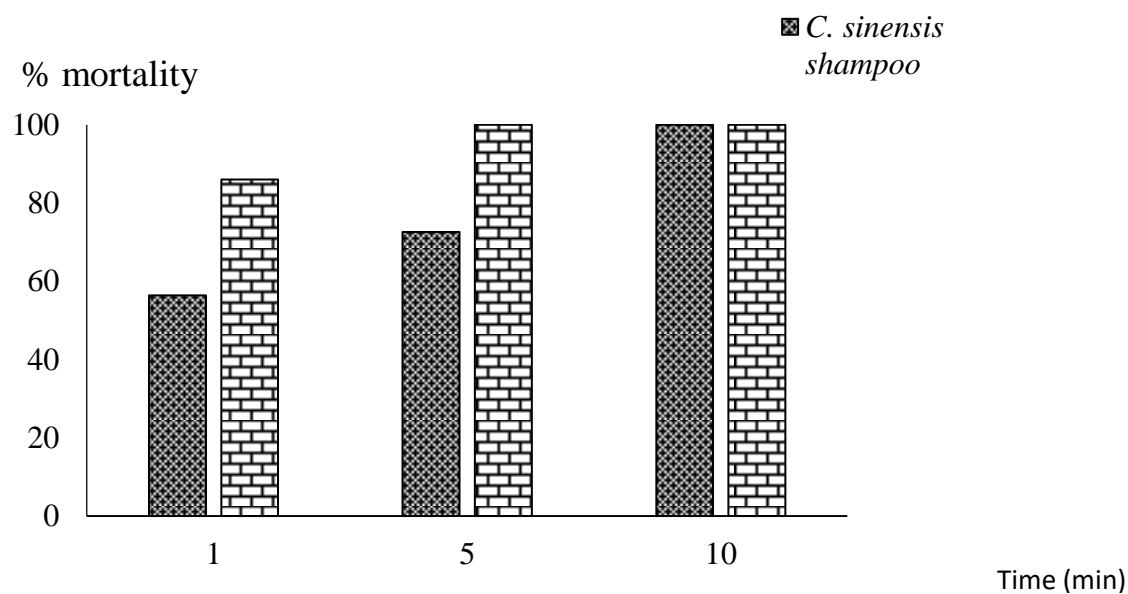


Figure 1. Toxicity of two herbal shampoos on mortality of head lice at 1, 5, 10 min

Discussion

The results of this study showed that *Z. officinale* shampoo was the most effective pediculicide against with 100% mortality at 5 min and LT_{50} values < 1.0 min. (ranged from 0.4 to 0.7 min), followed by *C. sinensis* shampoo showed (100% mortality at 10 min and LT_{50} values ranged from 1.2 to 1.4 min. Meanwhile, chemical pediculicide (positive control) and baby shampoo (negative control) showed non pediculicidal effect to head lice (100% of head lice survived during the observation periods). In addition, chemical pediculicide or chemical insecticide is very harmful for human health and toxic for children, because of children have less-developed immune systems and are more susceptible and sensitive to the toxic effect of chemical pediculicide (Abdel-Ghaffar and Semmler, 2007; Burkhart, 2004). However, lindane (positive control in this study) is an organochloride marketed in 1% concentration shampoo. This agent has been used to treat head lice for more than 50 years but recently came under increased scrutiny because of its toxic adverse effects (Eisenhower, 2012). Besides, lindane has neurotoxic properties similar to those of DDT and it is easily absorbed into adipose and neural tissue and has caused neurotoxicity and anemia in children and killing head lice by overstimulation of the parasite's central nervous system. Adverse events associated with lindane include in rare cases, death and neurologic effects include dizziness, seizures, headache and paresthesia (Burkhart, 2004; Eisenhower, 2012). Lindane resistance among head lice has been reported in several countries such as USA, UK, The Netherland and Panama (Eisenhower, 2012). In Thailand, lindane was one of the primary mode of head lice eradication for primary schoolchildren, although lindane was poor efficacy and very harmful for children health. Moreover, USA (California) and parts of Europe have banned the use of lindane because of toxicity and environmental effects (Arab *et al.*, 2011). On the other side, lindane was a common chemical pediculicide in Thailand used for head lice treatment of Thai children. For baby shampoo (Johnson's baby soft shiny shampoo[®], negative control), the results showed that non-toxic effect to head lice, 100% of head lice survived during the observation period. However, Johnson's baby soft shiny shampoo is commonly shampoo for Thai children, but cannot used for head lice control. The herbal shampoos base on *Z. officinale* and *C. sinensis* are suitable to be used as pediculicide for Thai schoolchildren. However, rhizome *Z. officinale* is a common condiment for various food and beverages. It has a long history of medicinal use dating back 2,500 years in China and India for conditions such as headaches, nausea, rheumatism and colds (Bhargava *et al.*, 2012). and *Z. officinale* is native plant of Southern Asia including Thailand. In addition, tea, *C. sinensis* is native to main land of China, South and Southeast Asia, but it is today cultivated across the world in tropical and

subtropical regions. The polyphenols from *C. sinensis* much evidence indicates that have various biological activities including antifungal, anti-inflammation, anti-mutagenic, anti-oxidative, anti-carcinogenic, antitumor, anti-diabetic effects, the lowering of plasma cholesterol and triglyceride levels and reduction of blood pressure and platelet aggregation in several system (Arab *et al.*, 2011; Sherwaniet *al.*, 2013). Therefore, *Z. officinale* and *C. sinensis* shampoos have been suggested as alternative products for head lice control because they are good and safe alternative due to their less toxicity to children than chemical pediculicides.

Acknowledgements

The author are highly grateful to Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand for providing financial assistance to carry out this study. Thanks are extended to all authorities of the primary school in Ladkrabang district ,Bangkok, Thailand ,where head lice materials were collected.

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(Received: 5 October 2015, accepted: 25 October 2015)