*In vitro pediculicidal activity of herbal shampoo base on Thai local plants against head louse (*Pediculus humanus capitis *De Geer)*

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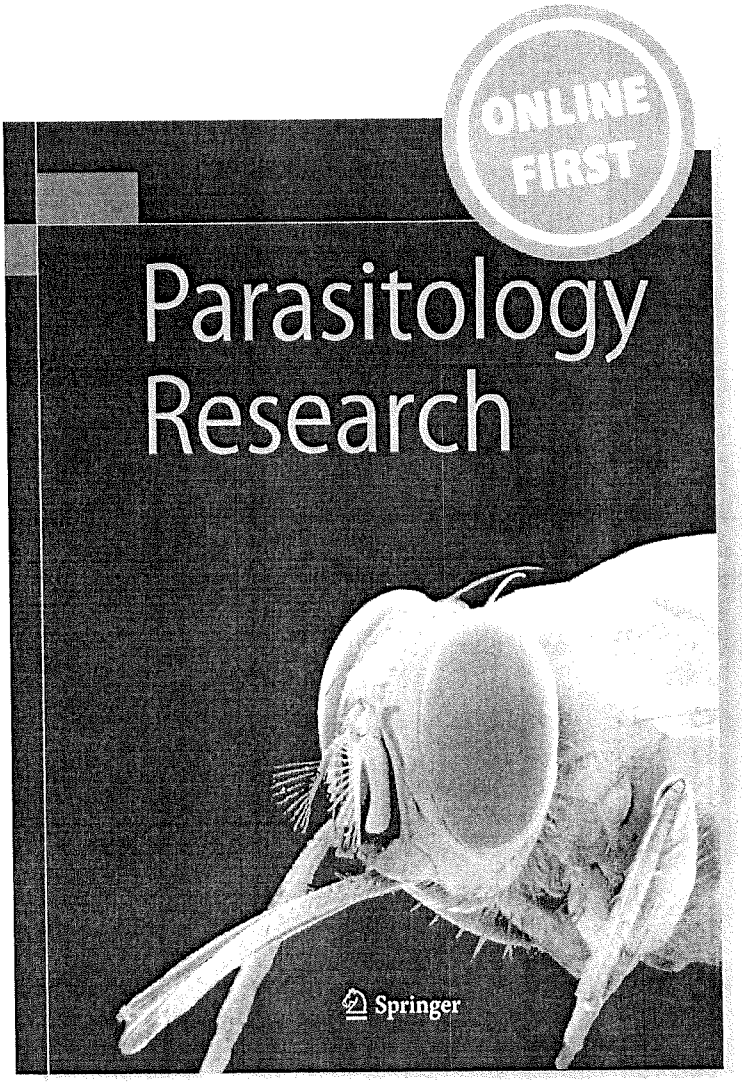

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ORIGINAL PAPER

In vitro pediculicidal activity of herbal shampoo base on Thai local plants against head louse (*Pediculus humanus capitis* De Geer)

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Abstract Head lice infestation, a worldwide head infestation caused *Pediculus humanus capitis* De Geer, is an important public health problem in Thailand. Several chemical pediculicides have lost in efficacy due to increasing resistance of lice against insecticide. Therefore, non-toxic alternative products, such as natural products from plants, e.g. plant extract pediculicides, are needed for head lice control. The aims of this study were to evaluate the potential of pediculicidal activity of herbal shampoo base on three species of Thai local plants (*Accacia concinna* (Willd.) DC, *Averrhoa bilimbi* Linn. and *Tamarindus indica* Linn.) against head lice and to compare them with carbaryl shampoo (Hafif shampoo®; 0.6 % *w/v* carbaryl) and non-treatment control in order to assess their in vitro. Doses of 0.12 and 0.25 ml/cm^2 of each herbal shampoo were applied to filter paper, and ten head lice were place on the filter paper. The mortalities of head lice on the filter paper were recorded at 1, 5, 10, 30 and 60 min by sterio-microscope. All herbal shampoos at 0.25 ml/cm^2 were more effective pediculicide than carbaryl shampoo with 100 % mortality at 5 min. The median lethal time (LT_{50}) of all herbal shampoos at 0.25 ml/cm^2 showed no significant differences over at 0.12 ml/cm^2 ($P<0.01$). The most effective pediculicide was *T. indica* extract shampoo, followed by *Av. bilimbi* extract shampoo and *Ac. concinna* extract shampoo, with LT_{50} values <1.0 min. Our data showed that all herbal shampoos have high potential of pediculicide to head lice treatments for schoolchildren.

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Introduction

Head lice (*Pediculus humanus capitis* De Geer; Phthiraptera: Pediculidae) are wingless insect and an ectoparasite that has been confined to the scalp and hair, live on the head and feed on the scalp of human for thousands of years (Araujo et al. 2000; Heukelbach et al. 2006a). The head lice infestations (*Pediculosis capitis*) are widespread throughout the world and more common in schoolchildren between the ages of 4–13 years and can be found in any sex, race, economic status, family background or social class in both developed and developing countries (Burgess 2004, 2009; Falagas et al. 2008; Mumcuoglu et al. 2009). The mode of transmission is most commonly via direct head-to-head contact and indirect transmission by sharing combs, brushes, caps, hats, pillows or other personal items of a person with head lice. However, head lice are not known to transmit infectious agents from person-to-person (Nutanson et al. 2008; Canadian Paediatric Society 2008). They feed by injecting saliva with vasodilatory properties into the scale to draw blood of human (Ko and Elston 2004). The clinical manifestations of head lice include pruritus, scalp impetigo, papules, excoriation, local erythema, cervical and occipital lymphadenopathy and chronic heavy infestation among schoolchildren may lead to anaemia (Frankowski et al. 2010; Ko and Elston 2004; Diamantis et al. 2009). Thus, head lice infestation cause not only physical symptoms but also psychological distress because children believe that head lice infestation is a result of being dirty (Oh et al. 2010). However, the number of head lice infestation case has increased worldwide since the mid-1960s, reaching hundreds of millions annually (Gratz 1997; Falagas et al. 2008). The high levels of infestations have also been reported from all over the world, ranging from 1.8 to 87.0 % (Burkhart and Burkhart 2006; Feldmeir 2012, Falagas et al. 2008; Gutierrez et al. 2012), and head lice infestation represented an important problem of public health.

The control of head lice worldwide (including Thailand) depends on chemical insecticides, such as organophosphate insecticides (malathion) and carbamate insecticides (carbaryl), despite that chemical insecticides are very harmful for human health and toxic for children. Children have less developed immune systems, underdeveloped detoxification mechanisms and more susceptible and sensitive to the toxic effect of chemical insecticides (Abdel-Ghaffar and Semmler 2007). Moreover, malathion has also been found to disrupt the immune system, and carbaryl is a potential human carcinogen (any substance that produce cancer) (Davis et al. 1993; Rassami and Soonwera 2011; Swadener 1992). Unfortunately, several topical head lice treatments base on chemical insecticides failed to obtain a head lice control, and increasing resistance of head lice against chemical insecticides have been reported in several countries (Burgess 2009; Burgess and Burgess 2011; Burkhart and Burkhart 2006; Hunter and Barker 2003; Heukelbach and Feldmeier 2004; Mumcuoglu et al. 2009).

Many plant-based products have been suggested as alternative products for head lice control because they are good and safe alternatives due to their less toxicity to human than chemical insecticides and easy biodegradability (Bagaven et al. 2011; Heukelbach et al. 2006a, b; Toloza et al. 2010a).

Plant-based compounds such as neem (*Azadirachta indica*), Henna (*Lawsonia inermis*), grapefruit, *Vitex agnus castus*, *Eucalyptus* sp., Tea tree oil (*Melaleuca alternifolia*), *Syzygium aromaticum*, *Melia azedarach*, *Curcuma longa*, *Zingiber montanum*, long pepper and bergamot essential oil have been taken into account for their activity against head lice and their nits, and could represent to confine the emergence and the spread of head lice infestation (Abdel-Ghaffar and Semmler 2007; Abdel-Ghaffar et al. 2010a, 2012; Bagaven et al. 2011; Campli et al. 2012; Carpinella et al. 2007; Gallardo et al. 2012; Greive and Barnes 2012; Marimuthu et al. 2012; Mehlhorn et al. 2011; Heukelbach et al. 2006b, 2008; Rassami and Soonwera 2011; Semmler et al. 2009, 2010; Soonwera et al. 2009; Toloza et al. 2010b; Yang et al. 2004)

Accacia concinna (Willd.) DC. *Averrhoa bilimbi* Linn. and *Tamarindus indica* Linn., belonging to the family Leguminosae, Oxalidaceae and Leguminosae, respectively, are common Thai local plants and have long been considered to have medicinal properties for human skin disease, anthelminite for round worm, expectorant, external itching and infected wound (Faculty of Pharmacy, Mahidol University 1992). Fruits of *Ac. concinna*, *Av. bilimbi* and *T. indica* are commonly used for several Thai foods. The biological activities of *Ac. concinna*, *Av. bilimbi* and *T. indica* extracts were shown to have insecticidal activity against head lice (Soonwera 2004)

The aims of this study were to evaluate the potential of pediculicidal activity of herbal shampoo base on three species of Thai local plants (*Ac. concinna*, *Av. bilimbi* and *T. indica*) against head lice and to compare them with carbaryl shampoo (Hafif shampoo®; 0.6 % *w/v* carbaryl) and non-treatment control in order to assess their in vitro.

Material and methods

Plant materials and herbal shampoo

The three species of Thai local plants were used in this study, as shown in Table 1. The three species of Thai local plants were identified, authenticated and submitted at Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. All herbal shampoos were provided by the medicinal plant laboratory, Faculty of Agricultural Technology, KMITL. All herbal shampoos were kept at room temperature before testing.

Chemical shampoo

Carbaryl shampoo (Hafif shampoo®; 0.6 % *w/v* carbaryl), a common chemical pediculicide in Thailand, was purchased from IDS Manufacturing Co. Ltd., Pathumthani province, Thailand and used as standard.

Head lice

Head lice (*P. humanus capitis*) were collected by dry combing from the head of 120 infested schoolchildren at a primary school in Ladkrabang, Bangkok, Thailand, in January–February 2012. After collection, head lice were transported to Entomological Laboratory, Faculty of

Table 1 List of Thai local plants, part used, location and active ingredient of herbal shampoo tested in this study

Scientific name, family	Part used	Location	Active ingredient
Accacia concinna (Willd.) DC, Leguminosae	Fruit	Chiangmai, Thailand	5, 10 % (w/v) crude extracts of *Ac. concinna* fruit
Averrhoa bilimbi Linn., Oxalidaceae	Fruit	Nakonratchasima, Thailand	5, 10 % (w/v) crude extracts of *Av. bilimbi* fruit
Tamarindus indica Linn., Leguminosae	Fruit	Nakonratchasima, Thailand	5,10 % (w/v) crude extracts of *T. indica* fruit

Table 2 Toxicity of herbal shampoo extracted from three species of Thai local plants and chemical shampoo against head lice at 0.25 ml cm^2

Herbal shampoo	LT_{50} (min)	(%) mortality/ time (min)			*P* value (vs negative control)
		1	5	10	
Ac. concinna extract shampoo	0.53	90.0	100	100	<0.05
Av. bilimbi extract shampoo	0.48	96.0	100	100	<0.05
T. indica extract shampoo	0.44	98.0	100	100	<0.05
Carbaryl shampoo (positive control)	1.83	48.0	96.0	100	<0.05
Negative control	0	0	0	0	

Agricultural Technology, KMITL. The protocol for head lice collection was approved by the head teacher of the primary school and in collaboration with school teachers.

Bioassay

In vitro tests were started within 30 min after collection of head lice. A filter paper contact bioassay was used to evaluate the toxicity and mortality of three herbal shampoos (*Ac. concinna* extract shampoo, *Av. bilimbi* extract shampoo and *T. indica* extract shampoo) and chemical shampoo (carbaryl shampoo) to head lice. Doses of 0.12 or 0.25 ml/cm^2 of each herbal shampoo were applied to the filter paper (Whatman®, No1); 5.0 cm in diameter). After drying for 30 s, each filter paper was placed on the bottom of a petri dish. Careful selection of ten head lice under a dissecting microscope was done, and ten head lice were placed on the filter paper. Negative control head lice were placed directly on the filter paper with water (without any treatment). Carbaryl shampoo (Hafit Shampoo®) was simultaneously run as a positive control. The mortalities of head lice on the filter paper were recorded under dissecting microscope at 1, 5, 10, 30 and 60 min. The criteria for mortality of head lice were defined as the complete absence of any vital signs such as gut movement, movement of antennae or movement of legs with or without stimulation using forceps (Heukelbach et al. 2006b, 2008). The criteria for pediculicidal activity of treatments were defined at the LT_{50} value<1.0 min (Soonwera et al. 2009). All treatments were replicated five times. The LT_{50} value was calculated by probit analysis (SPSS for Windows version 16.0).

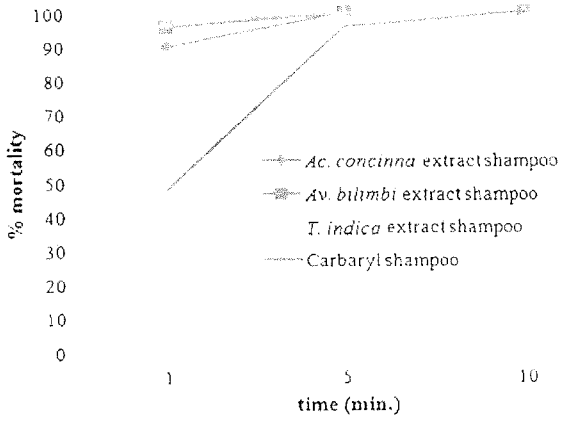

Fig 1 Pediculicidal activity of herbal shampoo extracted from *Ac. concinna*, *Av. bilimbi* and *T. indica* at 0.25 ml/cm^2 and carbaryl shampoo (positive control)

Results

The insecticidal activities of herbal shampoo from *Ac.concinna*, *Av. bilmibi* and *T. indica* at 0.25 ml/cm^2 against head lice were compared with the common chemical shampoo (carbaryl shampoo), as shown in Table 2. The mortality and LT_{50} values revealed that *T. indica* extract shampoo followed by *Av. bilmibi* extract shampoo and *Ac. concinna* extract shampoo were more toxic than carbaryl shampoo (LT_{50} value of 1.83 min) and LT_{50} values were 0.44, 0.48 and 0.53 min, respectively. Using the strict criteria for mortality, all head lice treated with all herbal shampoos did not show any vital signs, 100 % mortality at 5 min. After 10, 30 and 60 min, mortality was 100 % (Fig 1). All head lice in negative control group survived during the observation periods. In the carbaryl shampoo group (positive control), the mortalities were 48, 96, 100, 100 and 100 % at 1, 5, 10, 30 and 60 min, respectively (Fig 1). All herbal shampoos showed significant differences over negative control ($P<0.05$) and showed significant pediculicidal activity (LT_{50} values <1.0 min).

Ac. concinna extract shampoo, *Av. bilimbi* extract shampoo and *T. indica* extract shampoo were also toxic at 0.12 ml/cm^2 (Table 3). The pediculicidal activity was more pronounced in *T. indica* extract shampoo than *Av. bilimbi*

Table 3 Toxicity of herbal shampoo extracted from three species of Thai local plants and chemical shampoo against head lice at 0.12 ml/cm^2

Herbal shampoo	LT_{50} (min)	(%) mortality/ time (min)			*P* value (vs. negative control)
		1	5	10	
Ac. concinna extract shampoo	0.96	82.0	94.0	100	<0.05
Av. bilimbi extract shampoo	0.75	88.0	94.0	100	<0.05
T. indica extract shampoo	0.59	90.0	98.0	100	<0.05
Carbaryl shampoo (positive control)	1.87	50.0	94.0	100	<0.05
Negative control	0	0	0	0	

extract shampoo and *Ac. concinna* extract shampoo. All herbal shampoos (LT_{50} values of 0.53–0.96 min.) were more toxic than carbaryl shampoo (LT_{50} value of 1.87 min.); after 10, 30 and 60 min, mortality was 100 % (Fig 2). In the carbaryl shampoo group (positive control), the mortalities were 50.0, 94.0, 100, 100 and 100 % at 1, 5, 10, 30 and 60 min, respectively. For negative control, all head lice survived during the observation period. All herbal shampoos showed significant difference over negative control ($P<0.05$) and showed significant pediculicidal activity (LT_{50} values <1.0 min). However, the pediculicidal activity of all herbal shampoos at 0.25 ml/cm^2 showed no significant difference over pediculicidal activity at 0.12 ml/cm^2 ($P<0.01$), as shown in Table 4. Thus, pediculicidal assay indicted the order of pediculicidal activity in the herbal shampoos as *T. indica* extract shampoo>*Av. bilimbi* extract shampoo>*Ac. concinna* extract shampoo (Table 4).

Discussion

In this study, pediculicidal activity of herbal shampoos extracted from *Ac. concinna*, *Av. bilimbi* and *T. indica* were exhibited against head lice with LT_{50} value<1.0 min and 100 % mortality at 10.0 min. The LT_{50} values of herbal shampoos ranged from 0.50 to 0.96 min at 0.12 ml/cm^2 and LT_{50} values ranged from 0.44 to 0.53 min. at 0.25 ml/cm^2 and more effective than carbaryl shampoo (LT_{50} values of 1.83–1.87 min.). These LT_{50} values are in accordance with previous finding of other herbal shampoo toxicity against head lice (Rassami and Soonwera 2010; Soonwera et al. 2009).

On the basis of LT_{50} values, all herbal shampoos at 0.25 ml/cm^2 were more toxic to head lice than at 0.12 ml/cm^2, but LT_{50} values of all herbal shampoos showed no significant difference between 0.12 and 0.25 ml/cm^2. Therefore, the suggestion for the use of herbal shampoos as pediculicide, the effective dose is 0.12–0.25 ml/cm^2.

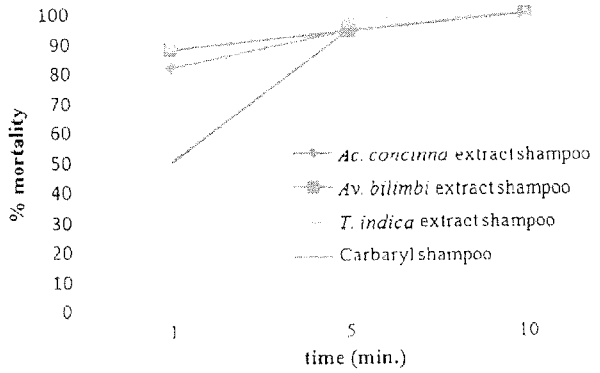

Fig 2 Pediculicidal activity of herbal shampoo extracted from *Ac. concinna*, *Av. bilimbi* and *T. indica* at 0.12 ml/cm^2 and carbaryl shampoo (positive control)

Table 4 LT_{50} values (min) of herbal shampoo extracted from three species of Thai local plants against head lice at 0.25 and 0.12 ml/cm^2

Herbal shampoo	LT_{50} (min) at 0.25 ml/cm^2	LT_{50} (min) at 0.12 ml/cm^2	*P* value
Ac. concinna extract shampoo	0.53	0.96	ns
Av. bilimbi extract shampoo	0.48	0.75	ns
T. indica extract shampoo	0.44	0.59	ns

ns not significant

Thus, herbal shampoos extracted from three species of Thai local plants (*Ac. concinna*, *Av. bilimbi* and *T. indica*) are suitable to be used as pediculicides for Thai schoolchildren because *Ac. concinna*, *Av. bilimbi* and *T. indica* are common plants in Thailand and have been extensively used for more than a thousand years in traditional Thai medicine. Dried fruit of *Ac. concinna* had been used for antidandruff and human skin disease, kernel seed of *T. indica* was used for anthelminite for roundworm, and the leaf and fruit of *Av. bilimbi* are used for expectorant, external itching and infected wound (Faculty of Pharmacy, Mahidol University 1992). However, since herbal shampoos claimed as natural products (Heukelbach et al. 2006a) are biodegradable, human and mammalian toxicity is considered low. Likewise, carbaryl shampoo (positive control) is toxic to human, and it also has been reported that carbaryl is a potential human carcinogen (any substance the produce cancer) (Davis et al. 1993; Swadener 1992). In addition, in vitro pediculicidal activity has been reported for some plant-based pediculicides such as Tea tree gel®, Lemon (*Citrus limon*), *Zingiber zeumbet* extract shampoo, *Zingiber officinale* extract shampoo and *Curcuma longa* extract shampoo (Heukelbach et al. 2006b, 2008; Shrivastava et al. 2010; Soonwera 2004). Rassami and Soonwera (2011) also reported herbal shampoo based on 10 % long pepper (*Piper retrofactum*) fruit extract which was shown to be highly effective against head lice infestations in clinical. It was recorded that more than 95.0 % mortality of head lice at 10.0 min after application of these herbal shampoos to infested schoolchildren of Ladkrabang area, Bangkok, Thailand as compared to 47 % mortality in the control group treated with commercial pediculicide. Long pepper has been used in traditional Thai medicine for a long time. Thus, long pepper extract shampoo is a highly effective treatment for head lice infestation; after the application of this herbal shampoo, it did not show any negative side effect such as erythema, skin irritation and burning sensation. The products from plant-based compound for commercial pediculicide such as Licatack shampoo® (extract s of grapefruit), Aesculo®Gel "L" (active compound noted, *Cocos nucifera* oil), Wash Away Laus shampoo (active compound noted, neem extracts), Picksan® Louse Stop Shampoo (active

compound noted, neem extracts). Nopucid Qubit® (active compound noted, geraniol, citronellol) and Nopucid Bio Citrus® (active compound noted, bergamot essential oil) showed to be highly effective against head lice, also reported by Abdel-Ghaffar et al. (2010a, b); Gallardo et al. (2012). Likewise, Abdel-Ghaffar et al. (2012) reported an anti-louse shampoo (Licener®) based on a neem seed extract which showed to be highly effective against head lice and their nits in vitro and in vivo. Thus, the product from plant-based compounds for anti-head lice has been developed for head lice treatment.

Therefore, the prevalence and degree of chemical insecticide resistance of head lice to pyrethrin, permethrin, malathion and carbaryl are expected to increase. Chemical insecticides are very harmful for human health and toxic for children. Alternative topical therapies for head lice infestation are needed. Thus, natural products, e. g. plant extract products, are safe alternative due to their less toxicity to human than chemical insecticides (chemical shampoo) and easy biodegradability. Possibly, on the long term, plant extract pediculicides are leading this growth and will replace chemical pediculicides on the markets. Finally, although herbal shampoos in this study showed to be highly effective in vitro against head lice, the important point should be tested for active ingredient of herbal shampoo acute and chronic toxicity in vivo clinical trails before using herbal shampoos as pediculicides for head lice treatments.

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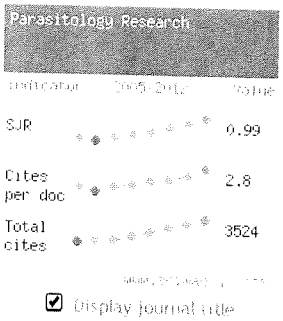

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