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Pediculicidal effect of herbal shampoo against *Pediculus humanus capitis in vitro*

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Abstract. Human head lice infestation is an important public health problem in Thailand. Lice resistance is increasing, chemical pediculicides have lost their efficacy and thus alternative products such as herbal shampoos have been proposed to treat lice infestation. The present study investigated the efficacy of twenty nine herbal shampoos based on zinbiberaceae plants, piperaceae plants and native plants against human head lice and compared them with malathion shampoo (A-lices shampoo®: 1% w/v malathion) and commercial shampoo (BabiMild Natural'N Mild®) in order to assess their *in vitro* efficacy. All herbal shampoo were more effective than commercial shampoo with 100% mortality at 60 seconds and LT_{50} values ranged from 11.30 to 31.97 seconds, meanwhile, commercial shampoo caused 14.0-15.0% mortality and LT_{50} values ranged from 83.96 to 87.43 seconds. The nine herbal shampoos from *Zingiber cassumunar*, *Piper betle*, *Piper ribesioides*, *Averrhoa bilimbi*, *Clitoria ternatea*, *Plectranthus amboincus*, *Myristica fragrans*, *Tacca chantrieri* and *Zanthoxylum limonella* were more effective pediculicide than malathion shampoo with 100% mortality at 30 seconds and LT_{50} values ranged from 11.30-13.58 seconds, on the other hand malathion shampoo showed LT_{50} values ranging from 12.39 to 13.67 seconds. LT_{50} values indicated the order of pediculicidal activity in the herbal shampoos as *Z. cassumunar* shampoo > *P. betle* shampoo > *Za. limonella* shampoo > *Av. bilimbi* shampoo > *P. ribesioides* shampoo > *My. fragrans* shampoo > *T. chantrieri* shampoo > *Pl. amboincus* shampoo. Our data showed that eight of the twenty nine herbal shampoos in this study were of high potential pediculicide to human head lice treatments for Thai children.

INTRODUCTION

Human head lice (*Pediculus humanus capitis* De Geer) is a small ectoparasitic insect that lives and feed on human beings for thousands of years (Araujo *et al.*, 2000; Heukelbach *et al.*, 2006; Intaranongpai *et al.*, 2006). Normally, human head lice transmission occurs by direct host-to-host contact (Takano-Lee *et al.*, 2005). Even though human head lice is not known to transmit diseases from person-to-person, however, their feeding activity irritates the scalp, and scratching can lead to secondary bacterial infections (Frankowski & Weiner, 2002; Gratz, 2006). It was found the average child with active pediculosis would lose 0.008 ml of blood per day (Speare *et al.*, 2006).

Moreover, human head lice infestation or *Pediculosis capitis* is a major public health problem throughout the world. Lice infestation has increased worldwide since the mid 1960s reaching hundred of millions annually, especially in schoolchildren between the ages 5-11 years. The high levels of lice infestations have also been reported from all over the world, ranging from 1.8 to 87.0% (Al-Shawa, 2008; Falagas *et al.*, 2008; Davarpanah *et al.*, 2009). In Thailand, human head lice infestation is a serious problem affecting more than 23.0% of the school-children in the eastern area of Bangkok

(urban area) and more than 80.0% of the schoolchildren in Ratchaburi province area (rural area) (Thanyavanich *et al*., 2009; Rassami & Soonwera, 2012).

However, the control of human head lice worldwide (including Thailand) depends on chemical insecticides such as organophosphate insecticides (malathion), organochlorine insecticide (lindane), carbamate insecticides (carbaryl), pyrethroid insecticides (pyrethrin). Regrettably, several of chemical insecticides failed to obtain lice control and increasing resistance of human head lice against chemical insecticides have been reported in several countries such as USA, England, Australia and Argentina (Burkhart & Burkhart, 2006; Burgess, 2009; Clark, 2009; Mumcuoglu *et al*., 2009; Sonnberg *et al*., 2010; Burgess & Burgess, 2011).

Thus, alternative topical therapies for human head lice infestations are needed, especially those containing plant-derrivative active ingredients. Furthermore, many of plant-based products have been suggested as alternative products for human head lice control because they are good and safe alternatives and easy biodegradability (Toloza *et al*., 2010; Bagavan *et al*., 2011). However, plant-based compounds such as *Azadirachta indica*, *Artemisia annua*, *Curcuma longa*, *Eucalyptus* sp, *Lawsonia inermis*, *Melia azedarach*, *Syzygium aromaticum*, essential oils from bergamot and tea tree have been taken into account for their activity against human head lice and their nits (Carpinella *et al*., 2007; Soonwera & Wangsapha, 2008; Soonwera *et al*., 2009; Toloza *et al*., 2010; Bagavan, 2011; Abdel-Ghaffar *et al*., 2012; Campli *et al*., 2012; Greive & Barnes, 2012). Consequently, twenty nine plants from zingiberaceae plants, piperaceae plants and native plants have been considered since they have medicinal properties in traditional Thai medicine as shown in Table 1–3.

The aim of this study was to evaluate the potential of pediculicidal activity of twenty nine herbal shampoos based on zingiberaceae plants, piperaceae plants and native plants against human head lice and compare them with malathion shampoo (A-Lice shampoo®; 1% w/v malathion) and commercial shampoo (BabiMild Natural'N Mild®) in order to assess their *in vitro* activity.

MATERIALS AND METHODS

Plant materials and herbal shampoos

Details of the twenty nine species from zingiberaceae plants piperaceae plants and native plants used in this study are shown in Table 1, 2, 3 and were indentified, authenticated and submitted to Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand. All of herbal shampoo were provided by the Medicinal Plant Laboratory, Faculty of Agricultural Technology, KMILT. The formulation of herbal shampoos are shown in table 1-3 and kept at room temperature before testing.

Chemical shampoo

Malathion shampoo (A-Lice shampoo®; 1.0% w/v malathion) is 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 standard.

Commercial shampoo

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

Human head lice

Human head lice were collected by a fine-toothed comb from the head of 780 infested schoolchildren 7-12 years old at 3 primary schools in Ladkrabang area, Bangkok, Thailand, during June – August, 2012. After collection, lice were transported to Entomological Laboratory, Faculty of Agricultural Technology, KMITL. The protocol for human head lice collection was approved by the head teacher of a primary school and in collaboration with school teachers.

Bioassay:
After collection of human head lice, *in vitro* tests were started within 30 min. Immersion test (Soonwera, 2004; Gallardo *et al.*, 2012) was used to evaluate the toxicity and mortality of twenty nine herbal shampoos, chemical shampoo (malathion shampoo, positive control) and commercial shampoo (negative control) to human head lice. 0.3 ml of each tested treatment were applied to a petri dish (5.0 cm in diameter). Careful selection of 10 human head lice under a dissecting microscope and 10 human head lice were immersed for 30 seconds. Once the exposure period finished, the lice were transferred onto a filter paper (Whatman® No1, 4.50 cm in diameter) moistened with 0.1 ml of water that was placed in the bottom of a petridish (5.0 cm in diameter) (Fig. 1b). The mortality of human head lice on the filter paper were recorded under dissecting microscope at 10, 15, 30 and 60 seconds. The criteria for mortality of human head lice were strict and were defined as absence of

Table 1. List of *Zingiberaceae* plants, part used and active ingredients of herbal shampoo tested in this study

Scientific name	Part used	Formualtion	Therapeutic property
Curcuma aeruginosa	rhizomes	10% (v/v) crude extract from *C. aeruginosa* rhizomes	antibacterial, anti-BPH, antiinflammatory, antioxidant, antipyretic.
C. aromatica	rhizomes	10% (v/v) crude extract from *C. aromatic* rhizomes	antioxidant, antiprurigo, carminative, stomachic.
Zingiber cassumunar	rhizomes	10% (v/v) crude extract from *Z. cassumunar* rhizomes	anticontusion, antiemetic, antiflatulence, antimicrobial, carminative, insecticide, insect repellent, spermicide.
Z. officinale	rhizomes	10% (v/v) crude extract from *Z. officinale* rhizomes	anesthesia, antibacterial, antidiabetic, antifungal, antineoplastic, antioxidant, antiviral, insecticide, nausea vomiting.
Z. ottensii	rhizomes	10% (v/v) crude extracts from *Z. ottensii* rhizomes	anticontusion, antidysentery, stimulant.
Z. zerumbet	rhizomes	10% (v/v) crude extract from *Z. zerumbet* rhizomes	antibacterial, carminative, stomachic, tonic.

Table 2. List of *Piperaceae* plants, part used and active ingredients of herbal shampoo tested in this study

Scientific name	Part used	Formualtion	Therapeutic property
Piper betle	leaf	10% (v/v) crude extract from *P. betle* leaves	antibactericidal, antihistaminic, carminative, local anesthetic, nematocide, stomachic.
P. nigrum	fruit	10% (v/v) crude extract from *P. nigrum* fruits	antiamoebic, antibacterial, antioxidant, antiyeast & mold.
P. retrofractum	fruit	10% (v/v) crude extract from *P. retrofractum* fruits	antidiarrhoea, antiseptic, antitussive, carminative, expectorant, preservative.
P. ribesioides	leaf	10% (v/v) crude extract from *P. argyrites* leaves	antiangina, antifungal, carminative, expectorant.
P. sarmentosum	leaf	10% (v/v) crude extract from *P. sarmentorum* leaves	antidysentery, appetizing, carminative, expectorant.

Table 3. List of native plants, part used and active ingredients of herbal shampoo tested in this study

Scientific name/Family	Part used	Formulation	Therapeutic property
Acacia concinna Leguminosae	fruit	10% (v/v) crude extract from *A. concinna* fruits	antidandruff, antipyretics, appetizing, expectorant, laxative.
Acorus calamus Araceae	rhizomes	10% (v/v) crude extract from *A. calamus* rhizomes	antihypertensive, carminative, expectorant.
Andrographis paniculata Acanthaceae	leaf	10% (v/v) crude extract from *A. paniculata* leaves	antidiarrhoea, antidysentory, antiinflammatory, antipyretics, expectorant.
Arcangelisia flava Menispermaceae	rhizomes	10% (v/v) crude extract from *A. flava* rhizomes	antidiarrhoea, antidysentery, antimalaria, antipyretics, appetizing, carminative, increase blood supply.
Averrhoa bilimbi Oxalidaceae	fruit	10% (v/v) crude extract from *A. bilimbi* fruits	antihemorrhoid, antipyretics, antitussive.
Av. carambola Oxalidaceae	fruit	10% (v/v) crude extract from *A. carambola* fruits	antidysentery, antiinflammatory, antipyretics, antitussive, expectorant, laxative.
Citrus aurantifolia Rutaceae	fruit	10% (v/v) crude extract from *C. aurantifolia* fruits	antiaphthous ulcer, carminative, expectorant, nematocide.
C. hystrix Rutaceae	fruit	10% (v/v) crude extract from *C. hystrix* fruits	antiamoebic, mosquito replant.
Clitoria ternatea Leguminosae	flower	10% (v/v) crude extract from *C. ternatea* flowers	anti hair loss, antioxidant, improve blood circulation.
Eupatorium odoratum Asteraceae	leaf	10% (v/v) crude extract from *E. odoratum* leaves	acceleration, antiinflamatory, staunch, wound healing.
Mentha arversis Meliaceae	fruit	10% (v/v) crude extract from *A. odorata* fruits/ pH 7.0	analgesic, antitussive.
Myristica fragrans Myristicaceae	fruit	10% (v/v) crude extract from *M. fragrans* fruits	antidiarrhoea, antispasmodic, carminative, tonic.
Nigella sativa Ranunculaceae	rhizomes	10% (v/v) crude extract from *N. sativa* rhizomes	antidiabetic, antihistaminic, antiinflammatory, antioxidant, antimicrobial, antitumor.
Phyllanthus emblica Euphorbiaceae	fruit	10% (v/v) crude extract from *P. emblica* fruits	antidiarrhoea, antipyretics, antitussive, expectorant, nourish the heart.
Plectranthus amboinicus Labiatae	leaf	10% (v/v) crude extract from *P. amboinicus* leaves	antipyretics, appetizing.
Polygonum odoratum Polygonaceae	leaf	10% (v/v) crude extract from *P. odoratum* leaves	antiflatulence, appetizing, carminative.
Tacca chantrieri Taccaceae	rhizomes	10% (v/v) crude extract from *T. chantrieri* rhizomes	antiinflammatory, antifeedant of larvae, antihypertensive.
Zanthoxylum limonella Rutaceae	fruit	10% (v/v) crude extract from *Z. limonella* fruits	carminative, increase blood supply, nourish the heart.

movement of limbs and gut, with or without stimulation using forceps (Campli *et al.*, 2012). The criteria for pediculicidal activity of treatments were defined at the LT_{50} value < 60 seconds (Soonwera, 2004; Soonwera & Rassami, 2011). All treatments were replicated ten times. The mortality data was analyzed with Duncan's multiple range test (DMRT) using SPSS for Windows version 16.0. The LT_{50} value was calculated using probit analysis.

RESULTS

The pediculicidal activity of six herbal shampoo from zingiberaceae plants, compared with malathion shampoo and commercial shampoo, is shown in Table 4. All human head lice treated with all of herbal shampoo showed 100% mortality at 60 seconds and LT_{50} values ranged from 11.30 to 22.97 seconds, on the other hand 86.0% of lice in negative control group survived during the observation periods. The most effective pediculicide was *Zingiber cassumunar* shampoo with 100% mortality at 30 seconds and LT_{50} value of 11.30 seconds, meanwhile malathion shampoo and commercial shampoo showed 100% and 2.0±4.22% mortality and LT_{50} values of 12.39 and 87.43 seconds, respectively. On the mortality and LT_{50} values indicated the order of pediculicidal activity in the herbal shampoo from Zingiberaceae plants as *Z. cassumunar* > *Z. officinale* > *C. aeruginosa* > *C. aromitica* > *Z. ottensii* > *Z. zerumbet*.

The results of the pediculicidal activity of eighteen herbal shampoos from native plants are shown in Table 5. The results showed that the five herbal shampoos from piperaceae plants were more toxic to human head lice than commercial shampoo with 100% mortality at 60 seconds and LT_{50} values ranged from 11.75 to 23.67 seconds, meanwhile 85% of lice in commercial shampoo group survived during the observation periods. The mortality rate of human head lice treated with *Piper betle*, *Piper sarmentosum* and *Piper ribesioides* shampoo ranged from 8.0 to 13.0% and 41.0 to 99.0% at 10 and 15 seconds, respectively. At 30 seconds, all herbal shampoo except *Piper nigrum* shampoo exhibited 100% mortality, on the other hand commercial shampoo caused 2.0±4.22% mortality. The pediculicidal activity was more pronounced in *P. betle* shampoo and *P. ribesioides* shampoo (LT_{50} values ranged from 11.75 to 13.24 seconds) than malathion shampoo (LT_{50} values of 13.31 seconds). On the mortality and LT_{50} values indicated the order of pediculicidal activity in the herbal shampoo from piperaceae plants as *P. betle*

Table 4. Toxicity of herbal shampoo extracted from six species of Zingiberaceae, chemical shampoo and commercial shampoo on mortality of human head lice

Type of the herbal shampoo	% mortality±SD/time (sec.)					LT_{50} (sec.)
	10	15	20	30	60	
Curcuma aeruginosa	7±4.8b[1/]	31±11.9d	77±21.1c	100a	100a	16.67
C. aromatica	2±4.2bc	40±12.4d	73±10.5c	100a	100a	17.14
Zingiber cassumunar	37±13.3a	88±12.2a	100a	100a	100a	11.30
Z. officinale	7±6.7b	77±8.2b	93±9.4ab	100a	100a	13.62
Z. ottensii	0c	3±4.8ef	27±4.8d	94±9.6b	100a	22.03
Z. zerumbet	0c	12±10.3e	30±13.3d	86±11.7c	100a	22.97
Positive control (Chemical shampoo)	33±8.23a	55±16.5c	83±12.52bc	100a	100a	12.39
Negative control (Commercial shampoo)	0c	0f	1±3.16e	2±4.22d	14±9.66b	87.43
CV(%)	60.04	28.03	18.62	6.54	3.82	

[1/] % 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 5. Toxicity of herbal shampoo extracted from five species of Piperaceae, chemical shampoo and commercial shampoo on mortality of human head lice

Type of the herbal shampoo	% mortality±SD/time (sec.)					LT_{50} (sec.)
	10	15	20	30	60	
Piper betle	10±6.6b[1/]	99±3.1a	100a	100a	100a	11.75
P. nigrum	0c	5±8.5e	20±8.1c	91±11.9b	100a	23.67
P. retrofractum	0c	0d	20±22.1c	100a	100a	22.38
P. ribesioides	8±9.1b	79±8.7b	99±3.1a	100a	100a	13.24
P. sarmentosum	13±10.5b	41±21.8c	94±5.1a	100a	100a	15.01
Positive control (Chemical shampoo)	31±7.38a	46±10.75c	85±7.07b	100a	100a	13.31
Negative control (Commercial shampoo)	0c	0d	0d	2±4.22c	15±7.07b	83.96
CV(%)	73.36	26.85	16.04	5.66	3.04	

[1/] % 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)

> *P. ribesioides* > *P. sarmentosum* > *P. retrofractum* > *P. nigrum*.

The results of the pediculicidal activity of eighteen herbal shampoos from native plants are shown in Table 6. Human head lice treated with all herbal shampoo caused 100% mortality at 60 seconds and LT_{50} values ranged from 12.02 to 31.97 seconds, while, commercial shampoo caused 15.0±7.07% and LT_{50} value of 86.26 seconds. At 30 seconds, herbal shampoos from *Averrhoa bilimbi*, *Averrhoa carambola*, *Plectranthus amboincus*, *Andrographis paniculata*, *Citrus hystrix*, *Citrus aurantifolia*, *Zanthoxylum limonella*, *Acorus calamus*, *Myristica fragrans*, *Mentha arversis*, *Acacia concinna*, *Polygonum odoratum* and *Tacca chantrieri* caused 100% mortality, followed by herbal shampoos from *Eupatorium odoratum*, *Nigella sativa*, *Phyllanthus emblica*, *Clitoria ternatea* and *Arcangelisia flava* caused 91.0±11.9, 88.0±7.8, 80.0±4.5, 75.0±5.2 and 50.0±21.6%, respectively. The top five herbal shampoos from Table 6 were *Za. limonella* shampoo, *Av. bilimbi* shampoo, *My. fragrans* shampoo, *T. chantrieri* shampoo and *Pl. amboincus* shampoo (LT_{50} values ranged from 12.02 to 13.58 seconds) and were more effective of pediculicide than malathion shampoo (LT_{50} value of 13.67 seconds).

DISSCUSSION

In this study, all herbal shampoo from zingiberaceae plants, piperaceae plants and native plants evaluated against human head lice showed 100% mortality at 60.0 seconds and LT_{50} values < 35.0 seconds and more effective pediculicide than commercial shampoo (BabiMild Natural'N Mild®). BabiMild Natural'N Mild® used as commercial shampoo in this study is commonly shampoo for Thai children, but can not used for human head lice control.

The top of twenty nine herbal shampoo in this study were herbal shampoos from *Z. cassumunar*, *P. betle*, *Za. limonella*, *Av. bilimbi*, *P. ribesioides*, *My. fragrans*, *T. chantrieri* and *Pl. amboincus* and were highly effective as pediculicide with 100% mortality at 30 seconds and LT_{50} values < 13.60 seconds, on the other hand, malathion shampoo exhibited LT_{50} values > 13.70 seconds. However, these mortality and LT_{50} values are in accordance with previous finding of other herbal shampoos toxicity against human head lice (Soonwera, 2004; Soonwera *et al*., 2009; Soonwera & Rassami, 2011). Furthermore, Soonwera & Wangsapha (2007); reported that *Z. cassumnar* shampoo was highly effective as pediculicide with 100% mortality at 10.0 min. and LT_{50} value of

Table 6. Toxicity of herbal shampoo extracted from eighteen species of native plant, chemical shampoo and commercial shampoo on mortality of human head lice

Type of the herbal shampoo	% mortality±SD/time (sec.)					LT_{50} (sec.)
	10	15	20	30	60	
Acacia concinna	0e[1/]	40±9.4ef	97±6.7a	100a	100a	15.65
Acorus calamus	1±3.16e	76±9.6ab	97±4.8a	100a	100a	13.98
Andrographis paniculata	2±4.2de	55±16.5cd	98±4.2a	100a	100a	14.77
Arcangelisia flava	1±3.1e	12±19.3hi	22±24.4e	50±21.6d	100a	31.97
Averrhoa bilimbi	10±8.1bc	82±23.4a	97±6.7a	100a	100a	13.14
Av. carambola	1±3.1e	53±20.5cde	98±4.2a	100a	100a	14.93
Citrus aurantifolia	0e	31±19.6fg	85±9.7b	100a	100a	16.95
C. hystrix	0e	31±15.2fg	86±8.4b	100a	100a	16.68
Clitoria ternatea	0e	30±13.2fg	60±24.1cd	75±4.1c	100a	20.90
Eupatorium odoratum	0e	28±7.8fg	78±23.0b	91±11.9b	100a	19.14
Mentha arversis	0e	52±14.1de	52±14.1d	100a	100a	17.54
Myristica fragrans	7±4.8cd	80±16.3a	98±4.2a	100a	100a	13.33
Nigella sativa	0e	0i	9±8.7f	88±7.8b	100a	26.28
Phyllanthus emblica	0e	20±10.2gh	54±11.2d	80±4.5c	100a	20.97
Plectranthus amboincus	13±8.2b	66±23.1bc	99±3.1a	100a	100a	13.58
Polygonum odoratum	2±4.2de	31±9.9fg	68±12.2c	100a	100a	22.57
Tacca chantrieri	3±4.8de-	86±13.5a	100a	100a	100a	13.47
Zanthoxylum limonella	31±19.1a	79±17.2ab	100a	100a	100a	12.02
Positive control (Chemical shampoo)	28±6.32a	42±10.33def	79±9.97b	100a	100a	13.67
Negative control (Commercial shampoo)	0e	0i	1±3.16f	3±4.83e	15±7.07b	86.26
CV(%)	115.36	31.54	13.08	6.60	1.65	

[1/] % mortality within the same column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple Range Test, $P<0.05$)

8.86 min. Rassami & Soonwera (2010) also reported that *Za. limonella* shampoo exhibited high pediculicidal activity and essential oil from *Za. limonella* was effective to control *Aedes albopictus* (Das *et al.*, 2003). In addition, *in vitro* pediculicidal activity has been reported for some plant-based pediculicide such as tea tree (*Melaleuca alternifolia*), lemon (*Citrus limon*), *C. longa*, *Annona squamosa*, *Canaga odorata*, *Cymbopogon nardus* and *Cymbopogon winteratus* (Soonwera, 2004; Heukelbach *et al.*, 2008; Rossini *et al.*, 2008; Shrivastava *et al.*, 2010).

Moreover, the herbal shampoo from *Z. cassumnar*, *P. betle*, *Za. limonella*, *Av. bilimbi*, *P. ribesiodes*, *My. fragrans*, *T. chantrieri* and *Pl. amboincus* are suitable to be used as pediculicides for Thai schoolchildren. Besides, rhizome of *Z. cassumnar* are used for anticontusion, antiemetic, antiflatulence, antimicrobial, carminative, insecticide and insect repellent. Leaves of *P. betle* and *P. argyrites* are used for antibacterial, antifungal, carminative and expectorant. Fruits of *Av. bilimbi*, *Za. limonella* and *My. fragrans* are used for antihemorrhoid, antipyretics, carminative and antidiarrheal. Leaves of *Pl. amboinicus* are used for antipyretic and appetizing, rhizome of *T. chantrieri* are used for antiinflammatory, antihypertensive, including fruit of *Av. bilimbi* and leaf of *Pl. amboinicus* are commonly used for several Thai foods (Faculty of Pharmacy, Mahidol University, 1992).

However, plant-based product have been suggested as an alternative products for human head lice control because they are good and safe alternatives due to their less toxicity to human than chemical pediculicides and easy biodegradability

(Heukelbach *et al*., 2006; Bagavan *et al*., 2011). Meanwhile, malathion has also been found to disrupt the immune system of human, thus malathion shampoo is very harmful for human health and toxic for children (Abdel-Ghaffar & Semmler, 2007). Therefore, new generations of anti-head lice products from plant-based compounds have been developed (Feldmeier, 2012). Furthermore, the commercial pediculicide products based on plants such as Licatack shampoo® (extracts of grapefruit), Aesculo Gel® "L" (active compound noted *Cocos nucifera* oil), Wash Away Laus shampoo® (active compound note, neem extracts), Nopucid Bio Citrus® (active compound noted, bergamot essential oil) showed high effectiveness against human head lice (Abdel- Ghaffar *et al*., 2010; Gallardo *et al*., 2012).

Finally, while an herbal shampoo in this study showed to be highly effective *in vitro* against human head lice, the important point the active ingredient should be tested for acute and chronic toxicity *in vivo* clinical trails before it is used as a herbal shampoo as pediculicides for human head lice treatments. In addition, the infestation with human head lice is a serious public health problem affecting schoolchildren in Thailand especially in Bangkok, the infestation rate > 20% (Rassami & Soonwera, 2012). The suggestion for human head lice eradication is for the parents and teachers to inform the infested schoolchildren to treat their hair with anti-head lice shampoo based on plant or herb shampoo at least 3-4 times a week for 1 month.

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