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Toxicity of Five Herbal Extracts against Head Louse (*Pediculus Humanus Capitis* De Geer.: Phthiraptera) in Vitro

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Abstract Head lice infestation caused by *Pediculus humanus capitis* De Geer. (Phthiraptera) is one of the most important public health problem in children worldwide. Currently, head lice resistance to chemical pediculicide are increasing, thus new alternative pediculicides from herb or plant are needed for head lice treatment. The aim of this study were to evaluate toxicity of five herbal extracts from *Andrographis paniculata* Wall ex Nees (Acanthaceae), *Arcangelisia flava* (L.) Merr. (Menispermaceae), *Butea superba* Roxb (Leguminosae), *Illicium verum* Hook.f. (Illiciaceae) and *Nigella sativa* Linn. (Ranunculaceae) on mortality of *P. humanus capitis* at dose of 3 and 6 µl/cm^2 by filter paper method in vitro. The results showed that all herbal extract at 6 µl/cm^2 was more toxic than 3 µl/cm^2 with LT_{50} values ranged from 0.1 to 21.8 h and 0.6 to 25.2 h, respectively. Moreover, the most toxic was shown by 6 µl/cm^2 of *I. verum* extract with 100%mortality at 12 h and LC_{50} value of 0.3 µl/cm^2, followed by extracts from *A. paniculata*, *N. sativa*, *B. superba* and *A. flava* with 82.6±6.9, 63.0±7.6, 47.0±9.7 and 32.0±5.7% mortality and LC_{50} values of 14.2, 20.3, 22.5 and 22.8 µl/cm^2, respectively. The data pointed that *I. verum* extract showed high toxic to head lice and showed high potential of pediculicide for head lice control.

Keywords: head lice, *Pediculus humanus capitis*, herbal extract, head lice control, *Illicium verum*

Introduction

Pediculosis capitis is an infestation of the human scalp caused by *Pediculus humanus capitis* (Phthiraptera: Pediculidae), generally known as head lice. However, these disease is a worldwide problem, especially primary school children, and estimated at 300 million case per year (Hunter and Barker,2003; Mumcuoglu *et al.*, 2009). The main symptoms of head lice infestation are severe pruritus on the head, scalp irritation, allergies, skin inflammatory, sleep loss and may lead to secondary bacterial infections, such as

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Bartonella quintana, Acinetobacter baumannii and *Borrelia recurrentis* (Bonilla *et al.*, 2009; Bouvress *et al.*, 2011). Moreover, pediculosis capitis can also cause psychological problems, social sanctioning, low self-esteem in infested children(Rutkauskis *et al.*, 2015) and head lice infested families are mobbed as dirty and unhygienic(Semmler*et al.*, 2010). However, the mode of transmission of head lice occurs mainly by direct host-to-host contact or close head-to-head contact(Takano-Lee *et al.*, 2005). Presently, chemical control of head louse has been performed using a wide variety of insecticides from lindane, malathion, carbaryl and synthetic pyrethroids. Regrettably, some chemical pediculicides are too toxic to human such as lindane and other chemical pediculicides have lost their full efficacy due to increasing resistances of head lice to chemical pediculicides have been reported world wide (Bouvress*et al.*, 2012 ; Burkhart, 2004). In addition, the high costs of chemical pediculicides for head lice treatment such as the cost-effectiveness for head lice treatments with malathion 0.5% or permethrin 1.0% was US $161.75 per cure(Gur and Schneeweiss, 2009) and in the USA, spending on pediculicidal treatment in the year 2000 was approximately US $1 billion, furthermore, to losses arising from school days and work days lost by infested children and family(Rutkauskis *et al.*, 2015). Therefore, new alternative pediculicides for head lice treatment are need, especially to develop new alternative pediculicide from plants or herbs, and these pediculicides are good candidates for safer control agents that may provide good anti-lice activity and low levels of evolved head lice resistance (Rossini *et al.*, 2008).However, several plant species with documented activity against head lice such as *Annona squamosal* (Annonaceae), *Cananga odorata* (Annonaceae), *Rosmarinus officinalis* (Lamiaceae), *Aniba rosaeadora* (Lauraceae), *Cinnamomum zeylanicum* (Lauraceae), *Azadirachta indica* (Meliaceae), *Eucalyptus globulus* (Myrtaceae) and *Cymbopogon nardus* (Poaceae)(Rossini *et al.*, 2008). In Thailand, head lice infestation was found to be the most common among children, especially severity of head infestation among girls, with infestation rate varied from 84.30% to 88.40% for rural area and more than 30% for urban area(Rassami and Soonwera, 2012; Soonwera, 2014). Moreover, chemical insecticides used for control of head lice in Thailand were lindane, malathion, carbaryl and permethrin and some chemical insecticides such as lindane and malathion are too toxic to Thai children. Thus, this study aims to test efficacy of five herbal extracts from *Andrographis paniculata* (Acanthaceae), *Arcangelisia flava* (Menispermaceae), *Butea superba* (Leguminosae), *Illicium verum* (Illiciaceae) and *Nigella sativa* (Ranunculaceae) against head lice (*P. humanus capitis*) in vitro. However, all herb in this study has been used for therapeutic purposes for at least 1,000 years and all herb is the basis of traditional medicine in Asia

including Thailand (Table 1) (Ingkaninan *et al.*, 2003 ; Malaivijitnond *et al.*, 2009; Faculty of Pharmacy, Mahidol University, 1992).

Table 1. List of five species from herbs, part used and therapeutic property in this study

Scientific name Family	Part used	Therapeutic property
***Andrographis paniculata* Wall ex Nees F. Acanthaceae**	leaf & stem	Antidiarrheal, antidysentory, anti-inflammatory, antipyretic, bitter tonia and remedy for sore throat
***Arcangelisia flava* (L.) Merr. F. F.Menispermaceae**	stem	Stomachic, blood tonic, emmenagogue, antidiarrheal and carminative
***Butea superba* Roxb F. Leguminosae**	root	Acetylcholinesterase inhibitor, neurotonic agent, rejuvenating agent, anti-oxidants, anti-proliferation
***Illicium verum* Hook.f. F. Illiciaceae**	fruit	Carminative, stimulant, antifungal, antibacterial and anti-inflammatory
***Nigella sativa* Linn. F. Ranunculaceae**	seed	Antioxidant, antitumor, anti-inflammatory and antidiabetic

Materials and methods

Plant materials

The leaves and stems of *Andrographis paniculat(*Acanthaceae), stems of *Arcangelisia flava(*.Menispermaceae), roots of *Butea superba* (Leguminosae), were collected from Nakhornratchasima province, Thailand and fruits of *Illicium verum* (Illiciaceae)and seed of *Nigella sativa(* Ranunculaceae)were collected from traditional medicine store at Bangkok, Thailand, during April to May 2014. All herb identification was made by plant taxonomist from Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The voucher specimens were numbered and kept in laboratory of plant science, KMITL.

Preparation of plant extracts

Various parts of five herbs (2.1) were cleaned and air-dried for 7 days in the side at the laboratory temperatures (31.5±2.5°C, day time). The 1,000 g of dried and powdered of each herb was soaked in 2,000 ml of 95% ethyl alcohol at room temperatures for 5 days. The crude extract of each herb was

separated by suction filtered, and the filtrate was concentrated to dryness with rotary evaporated. Each herb extract was kept in closed bottle and placed in the refrigerator at 5 and 10% concentrations by dissolving in distilled water, which was used for the bioassays.

Collection of head lice

The protocol for head lice collection was approved by the director for the four primary schools located in Ladkrabang area, Bangkok 10520, Thailand and in collaboration with primary school teachers and parents of primary school children. However, the schoolchildren had not been treated with any chemical or herbal pediculicides for preceding month and allowed using only the fire-toothed comb. The collection of head lice were collected from 250 selected schoolchildren between ages of 5 to 7 years by raking a plastic fire-toothed comb through sections of the children's scalp. Afterwards, head lice were obtained and pooled by removing them from teeth of the comb into clean insect boxes (18x23x5.5 cm), during May to July, 2014. All head louse were transported to Laboratory of Entomology, Faculty of Agricultural Technology, KMITL. The head louse specimens were kept in Laboratory of Entomology, KMITL, for further reference.

Bioassay

The in vitro tests at Laboratory of Entomology, KMITL were started within 15 min, after collection of head lice, by filter paper contact toxicity bioassay (Soonwera, 2014) at doses of 3 and 6 µl/cm^2. Each dose of herbal shampoo was applied to the filter paper 4.5 cm in diameter. After drying of each filter paper for 30 s, each filter paper was placed on the bottom of a petri dish (5.0 cm in diameter). The ten of head lice adults were placed on each bottom of a petri dish. Negative control, head lice were placed directly on the filter paper with distilled water (without any treatment). All treated and control head lice were held at the same condition (33.5±2.3°C and 65.5±4.0% relative humidity (RH). The mortality of head lice were recorded at 0.5, 1.0, 6.0, and 12.0 h, under stereomicroscope. However, mortality criteria of head lice were according to Soonwera (2014). All treatments were replicated 10 times. The Median Lethal Time (LT_{50} value) and Median Lethal Concentration (LC_{50} value) were calculated by probit analysis (SPSS for Windows version 16.0). However, data of adult mortality was analyzed with Duncan's Multiple Range Test (DMRT).

Results

The effect of fie herb extracts on mortality of head lice (*P. humanus capitis*) at 3 µl/cm^2 and 6 µl/cm^2 as shown in Table 2 and Table 3, respectively. However, LT_{50} values in minutes and LC_{50} values in µl/cm^2 of five herb extracts against head lice is presented in Table 4. All head lice treated with all herbal extract at dose of 3 µl/cm^2 showed 0 to 58.0±8.4%, 7.6±2.5 to 82.0±9.1, 7.6±2.5 to 97.0±4.5 and 10.6±4.4 to 100% mortality at 0.5, 1, 6 and 12 h, respectively and LT_{50} values ranged from 0.6 to 25.2 h. Meanwhile, 100% of head lice in control (untreated) survived during 12 h (experiment periods). However, the most toxic was shown by *I. verum* extract with 100% mortality of head lice at 12 h and LT_{50} value of 0.6 h, followed by extracts from *A. paniculat, N. sativa*, *B.superba* and *A. flava* with 75.6±7.1, 57.0±7.3, 35.0±6.6 and 10.6±4.4% mortality at 12 h and LT_{50} values of 8.9, 11.4, 20.3 and 25.2 h, respectively. However, all herbal extract at high dose (6 µl/cm^2) showed high toxic to head lice, all head lice treated with all herbal extract at dose of 6 µl/cm^2 showed 2.0±1.7 to 83.0±12.1, 11.4±2.2 to 87.0±6.7, 23.2±4.3 to 100 and 32.0±5.7 to 100% mortality at 0.5, 1, 6 and 12 h, respectively and LT_{50} values ranged from 0.1 to 21.8 h. Moreover, the most toxic was also shown by *I. verum* extract with 100% mortality of head lice at 12 h, LT_{50} value of 0.1 h and LC_{50} value of 0.3 µl/cm^2, followed by extracts from *A. paniculat*, *N. sativa*, *B.superba* and *A. flava* with 82.6±6.9, 63.0±7.6, 47.0±9.7 and 32.0±5.7% mortality, LT_{50} values of 7.3, 10.7, 14.5 and 21.8 h and LC_{50} values of 14.2, 20.3, 22.5 and 22.8 µl/cm^2, respectively. All head lice in control (untried) survived during 12 h. In addition, on the mortality, LT_{50} values and LC_{50} values indicated the order of toxicity to head lice as extract from *I. verum* > *A. paniculat* > *N. sativa* > *B.superba* > *A. flava*.

Table 2. Effect of five herbal extracts on mortality of head lice (*P. humanus capitis*) at 3 µl/cm^2 concentration by filter paper method.

Treatment	% Mortality ± SD/time (h)			
	0.5	1.0	6.0	12.0
A. paniculat extract	12.6±4.9a[1/]	20.0±3.5b	28.0±7.6b	75.6 ±7.1b
A. flava extract	0c	0c	7.6±2.5c	16.6±4.4e
B.superba extract	0c	7.6±2.5bc	15.0±5.0bc	35.0±6.6d
I. verum extract	58.0±8.4b	82.0±9.1a	97.0±4.5a	100a
N. sativa extract	0c	7.6±2.5bc	20.0±3.5b	57.0±7.3c
distilled water (untreated)	0c	0c	0d	0f

[1/] Percent mortality within the same column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's Mutiple Rang test, $p<0.05$).

Table 3. Effect of five herbal extracts on mortality of head lice (*P. humanus capitis*) at 6 µl/cm^2 concentration by filter paper method.

Treatment	% Mortality ± SD/time (h)			
	0.5	1.0	6.0	12.0
A. paniculat extract	22.4±5.6b[1/]	35.6±7.1b	49.4±7.5b	82.6 ±6.9b
A. flava extract	2.0±1.7c	15.0±5.5c	23.2±4.3c	32.0±5.7d
B.superba extract	3.8±1.8c	11.4±2.2c	25.0±6.1c	47.0±9.7d
I. verum extract	83.0±12.1a	87.0±6.7a	100a	100a
N. sativa extract	4.4±3.7c	17.6±5.6c	31.0±9.6bc	63.0±7.6c
distilled water (untreated)	0d	0d	0d	0e

[1/] Percent mortality within the same column, followed by the same letter are not significantly different (one-way ANOVA and Duncan's Mutiple Rang test, $p<0.05$).

Table 4. Median lethal time values (LT_{50} values) in hour and median lethal concentration values (LC_{50} values) in µl/cm^2 of five herbal extracts on mortality of head lice (*P. humanus capitis*).

Treatment	LC_{50}[1/] values in µl/cm^2 at 1 h	LT_{50}[2/] values in hour at 3 µl/cm^2	LT_{50} values in hour at 6 µl/cm^2
A. paniculat extract	14.2	8.9	7.3
A. flava extract	22.8	25.2	21.8
B.superba extract	22.5	20.3	14.5
I. verum extract	0.3	0.6	0.1
N. sativa extract	20.3	11.4	10.7
distilled water (untreated)	ns[3/]	ns	ns

[1/] LC_{50} values = median lethal concentration, [2/] LT_{50} values = median lethal time
[3/] ns = not computed by Probit analysis

Discussion

The data in this study pointed that *I. verum* extract was the most toxic to head lice with 100% mortality at 6 h and LC_{50} value of 0.3 µl/cm^2, followed by extracts from *A. paniculat*, *N. sativa*, *B.superba* and *A. flava* with LC_{50} values of 14.2, 20.3, 22.5 and 22.8 µl/cm^2, respectively. However, *I. verum* extract showed high potential of pediculicide for head lice control and previous research reported insecticidal activity of *I. verum* against insect pest such as fruit flies (*Drosophila melanogaster*), *Blattella germanica*, *Lasioderma serricorns*, *Callosobruchus chinensis*, *Tribolium castaneum* and *Sitophilus oryzae* (Wang *et al.*, 2011; Shukla *et al.*, 2009; Kim *et al.*, 2003). In addition, Sinthusiri and Soonwera (2013; 2014) reported that 10% of *I. verum* essential oil gave 100% knockdown after exposure of 60 min to adult of house fly

(*Musca domestica*), with KT_{50} value of 18.66 min and LC_{50} value of 9.48% and 10% of *I. verum* essential oil caused 100% effective repellency against oviposition of house fly and oviposition activity index (OAI) = 1.0. Sripongpun (2008) also reported that crude extracts of *I. verum* fruits showed high toxic to larvae of house fly and their development in pupal and adult stages. Zhang(2012) reported that essential oil from *I. verum* fruit was highly toxic to larval and adult stage of *Dermestes maculatus* (museum insect pest) and Kimbaris *et al.* (2012) also reported that the essential oil from *I. verum* fruit showed larvicidal activity against larvae of *Culex pipiens*. However, *I. verum*, commonly called star anise, star aniseed, or Chinese star anise, belonging family Illiciaceae, is an aromatic evergreen tree bearing purple-red flowers and anise-scented star-shaped fruit. It grows almost exclusively in Southern China, Vietnam and Asia(Wang *et al.*, 2011),and this plant is well known in Thailand as "Chan paet Kleep"(Putiyanan *et al.*, 2010). Moreover, *I. verum* has been used for a few hundred years as a traditional Chinese herbal medicine to treat vomiting, stomach aches, insomnia, skin inflammation and rheumatic pain and essential oil of *I. verum* is used topically for rheumatology studies demonstrated that its crude extracts and active compounds possess wide pharmacological actions, especially in antimicrobial, antioxidant, insecticidal, analgesic, sedative and convulsive activities, and it is the major source of shikimic acid, a primary ingredient in the antiflu drug (Tamiflu)(Wang *et al.*, 2011). In Thailand, riped fruit of *I. verum* used for spice, stimulant and carminative(Faculty of Pharmacy, Mahidol University, 1992). However, do not confuse Chinese star anis (*I. verum*) with Japanese star anise (*Illicium anisatum*), *I. anisatum* is highly toxic and inedible, in Japan, it has instead been burned as incense. The toxicity of *I. anisatum*, also known as shikimi, is caused by its containing potent neurotoxins (anisatin, neoanisatin, and pseudo anisatin), due to their activity as noncompetitive antagonists of GABA receptor(Wikipedia, 2015). Currently, in rural and urban areas of Thailand, children are habitual to control head lice with chemical pediculicides such as lindane (Hexin lice killer Cream®; 1 % w/v Lindane), malathion (A-Lice shampoo®; 1.0% w/v malathion), carbaryl (Hafif shampoo®, 0.6 %w/v carbaryl) and permethrin (Scully Anti-Lice shampoo®, 0.5% w/w Permethrin), they are harmful to children, because of children have less developed immune systems, underdeveloped detoxification mechanisms(Abdel-Ghaffar and Semmler, 2007). Lindane has neurotoxic properties similar to those of DDT, killing head lice by overstimulation of the parasite's central nervous system(Burkhart, 2004). Furthermore, malathion resistance of head lice have been reported in Europe, and malathion is an organophosphate insecticide acting as a cholinesterase inhibitor and neurotoxic, it has also been found to disrupt the immune system(Abdel-Ghaffar and

Semmler, 2007). However, head lice resistance to carbaryl and permethrin have been reported in several countries. In November 1995, the UK government's Committee on carcinogenicity concluded that carbaryl is a potential human carcinogen (Abdel-Ghaffar and Semmler, 2007; Soonwera, 2014). Currently, 1% permethrin is considered by many to be the preferred first-line treatment for head lice infestations and permethrin works by paralyzing and killing head lice by disrupt neuronal sodium channels in nervous system of head lice (Narahashi *et al.,* 1998). Unfortunately, most of the head lice collected in the United States were not killed by permethrin, and the dose-response curve was not significantly different from zero (P=0.66) (Pallack *et al.,* 1999). Therefore, *I. verum* extract have great potential for the control of head lice, it is harmless to children and biodegradable, and in fact *I. verum* has long been used for herbal medicine and spice. All of these factors *I. verum* extract is a good alternative candidate for head lice treatment of schoolchildren in Thailand.

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