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Insecticidal activity of *Citrus aurantium* and *Eucalyptus globulus* essential oils and their major constituents against nymphs and adults of *Pediculus humanus capitis* De Geer

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Abstract The results showed that every tested EO and its constituents exhibited a higher pediculocidal activity against nymphs and adults of *P. humanus capitis* than 25% (w/v) benzyl benzoate did. In particular, 5% 1,8-cineole exhibited the highest insecticidal effects against *P. humanus capitis* nymphs and adults with 100% mortality rate at 1 min with an LT_{50} of 0.2 min and an LC_{50} of 0.7%. The other EO formulations provided a mortality rate ranging from 0-96.0% and an LT_{50} ranging 0.3-315.9 min against nymphs. Against the adults, the mortality rate ranged from 12.0-96.0% with an LT_{50} ranging from 1.2-314.5 min. Our data showed that 1,8-cineole has a great potential to control head lice populations and should be further developed into a safe and effective pediculicide.

Keywords: *Pediculus humanus capitis*, *Citrus aurantium* EO, *Eucalyptus globulus* EO, D-limonene, 1,8-cineole, Pediculicides

Introduction

The most common ectoparasitic disease in humans, head lice *Pediculus humanus capitis* De Geer infestation is most common in children aged 3 to 11 worldwide (Yang *et al*., 2004). A high prevalence up to 50% was found in developing countries and tropical countries, including Thailand (Baghdadi *et al*., 2021; Singhasivanon *et al*., 2019). Direct head-to-head contact between people can cause the transmission, as can indirect contact through the sharing of personal items like a brush, scarf, or hat (Yones *et al*., 2016). Although lice infestation is asymptomatic, the mainly well-known side effect are pruritus as well as skin irritation by the saliva. It can lead to abrasion and secondary infection from scratching (Yones *et al*., 2016; Yingklang *et al*., 2022).

Synthetic chemical pediculicides (e.g., benzene hexachloride, carbaryl, permethrin, ivermectin, and malathion) have long been used to control head

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louse infestation (Soonwera *et al*., 2018). Unfortunately, these pediculicides are exceedingly harmful to humans and extremely persistent. Additionally, it is widely known that populations of head lice become resistant to synthetic pediculicides (Yones *et al*., 2016). Plant-derived essential oils (EOs), e.g., *Callistemon viminalis*, *Cinnamomum aromaticum*, *Eucalyptus sideroxylon*, *E. globulus*, *Pimpinella anisum*, and *Sesamum indicum* have already been used as natural alternatives to synthetic pediculicides (Toloza *et al*., 2010; Yones *et al*., 2016; Soonwera *et al*., 2018; Yingklang *et al*., 2022). The advantages of EOs are that they are easily biodegraded, lower or non-toxic against mammals, and very effective against a wide spectrum of insect pests (Toloza *et al*., 2010; Yones *et al*., 2016).

Several researchers have reported the insecticidal activity of *Citrus aurantium* and *Eucalyptus globulus* and their major compositions against mosquito (*Anopheles labranchiae*), house fly (*Musca domestica*) (Dosoky and Setzer, 2018), and head lice (Yang *et al*., 2004; Sittichok and Soonwera, 2018). In addition, these EOs have been used as folk medicine for humans with efficacies including anti-microbial and anti-oxidant (Barbosa *et al*., 2016; Dosoky and Setzer, 2018).

This study aimed to determine the insecticidal activity of *Citrus aurantium* and *Eucalyptus globulus* EOs and their major constituents against nymphs and adults of *Pediculus humanus capitis* De Geer.

Materials and methods

Ethical statement concerning head lice collection

The human ethics committee at the Institute for Development of Human Research Protection (IHRP), Bangkok, Thailand, gave its approval to the methodology for collecting adult and nymph head lice from humans (permission number 76-2558).

Collection of head lice samples

Nymphs and adults of head lice were collected from the heads of 60 severely infested children—60 girls between 8-12 years old—who were students at several primary schools in Chachoengsao province, Thailand. Nymphs and adults were carefully removed from the teeth of lice combs and separated from one another into clean boxes (18.0 × 23.0 × 5.5 cm) according to the method of Soonwera *et al*. (2018). More than three hundred head lice were collected each time. Each stage of head lice was observed under a

stereomicroscope (Nikon® Type 102) within 15-20 min after the collection and separated from one another. The lice were kept under laboratory conditions at 27±4 ℃ and 74±4% relative humidity until the assay started.

Plant materials and essential oils

Fruits of *C. aurantium* and fresh leaves of *E. globulus* were collected during May-June 2019 from a farm in Nakhonratchasima province (14°57' 24" N / 102°5' 22" E) in the North-eastern part of Thailand. Specimens of the two plants were positively identified by a plant taxonomist from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. These plant pieces were used to extract essential oils (EOs) using a 5-hour hydro-distillation process. Following distillation, EOs were removed from the separating funnel, placed in airtight containers, and maintained at 4 ℃ for later tests. For insecticidal activity assay, the stock solution was diluted to 1% and 5% EO solutions in ethyl alcohol.

Chemicals

In this study, both a positive and a negative control were used. The positive control was 25% (*w/v*) benzyl benzoate, a popular pediculicide used to treat head lice infestations. It was manufactured by Vidhyasom Co., Ltd. (Bangkok, Thailand). The negative control was an aqueous solution of ethyl alcohol at 70% (*v/v*). It was manufactured by T.S Interlab Limited Partnership, (Bangkok, Thailand). D-limonene and 1,8-cineole, the major constituents of *C. aurantium* and *E. globulus* were purchased from Sigma-Aldrich (St. Louis, USA).

Insecticidal activity test

In vitro immersion test was started within 30 min after head lice were collected (Soonwera *et al*., 2018). A volume of 1,000 µl of each essential oil formulation was dropped onto a 5-cm diameter petri dish. Ten head lice nymphs or adults were immersed in that volume for 1 min. After the period of exposure, the head lice were dried on a Whatman® No. 1 filter paper (3 × 4 cm). The positive and negative controls were 25% (w/v) benzyl benzoate and 70% (v/v) ethyl alcohol run, respectively. The mortality of head lice on the filter paper was observed and recorded under stereomicroscope at 1, 10, 30, and 60 min. The strict criterion for head lice mortality was the complete absence of any vital signals, such as stomach movement, antennal movement,

or leg movement when stimulated with forceps (Sittichok and Soonwera, 2018). The experiments was done using completely randomized design with ten replicates for each treatment.

Statistical analysis

Probit analysis was used to determine the LT_{50} (50% Lethal Time) and LC_{50} (50% Lethal Concentration) values. One-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used in a statistical study of mortality data at a *P*-value of 0.05. SPSS Statistical Software Package, version 23.0, was used for all statistical analyses.

Results

The toxicity (mortality rate and LT_{50}) of every tested EO and EO constituent against *P. humanus capitis* nymphs for 1-min immersion time are tabulated in Table 2. When compared to the similar formulation with 1% EO and EO constituent, every formulation with 5% EO or 5% EO constituent demonstrated a significantly better efficacy. The high mortality rate, 48.0-100% at 60 min, that they provided were higher than that provided 25% (*w/v*) benzyl benzoate (positive control) (28.0% mortality rate and an LT_{50} of 223.8 min). Among the tested formulations, 5% d-limonene and 5% 1,8-cineole were more toxic against nymphs of head lice than the other formulations. They provided 100% mortality rate at 1 min and an LT_{50} of 0.2 min, while other formulations showed a mortality rate ranging from 0-96.0% and an LT_{50} ranging from 0.3 to 351.9 min. However, no head lice nymphs were killed by the negative control (70% *v/v* ethyl alcohol). Furthermore, both EO constituents, d-limonene and 1,8-cineole exhibited a strong insecticidal activity (LC_{50}) against head lice with no significant difference (Table 1).

The efficacy of every EO and EO constituent against *P. humanus capitis* adults, after 1-min immersion time, are summarized in Table 3. Among the tested formulations, 5% d-limonene and 5% 1,8-cineole were found to be the most effective with 100% mortality rate at 30 min and an LT_{50} of 1.2 and 0.2 min, respectively. Other formulations had mortality rate between 12.0-96.0% and an LT_{50} between 1.6 to 314.5 min. Conversely, 70% (*v/v*) ethyl alcohol (negative control) had no effect on the mortality of adult head lice at all, while 25% (*w/v*) benzyl benzoate (positive control) had a 36.0% mortality rate at 60 min and an LT_{50} of 35.0 min. The LC_{50} of 1,8-cineole was the lowest (most effective) against head lice adults (Table 1).

Table 1. Lethal concentration for 50% mortality (LC_{50}) against nymphs and adults of *P. humanus capitis* at 10 min of exposure to individual EO and EO constituent from *C. aurantium* and *E. globulus* EOs

Treatment	LC_{50} (%) (LCL-UCL)	
	nymph	adult
C. aurantium EO	5.0 (3.5-6.9)	3.8 (0.1-7.3)
E. globulus EO	7.6 (-)	3.9 (2.5-5.6)
1,8-cineole	0.7 (0.1-0.9)	0.7 (0.2-1.0)
d-limonene	0.5 (-)	1.8 (1.5-4.3)

LC_{50} = 50% lethal concentration; *UCL* is upper confidence limit; *LCL* is lower confidence limit.

Table 2. Mortality rate and LT_{50} of essential oils from *C. aurantium* and *E. globulus* and their major constituents against nymphs of *P. humanus capitis*, for an immersion time of 1 min

Treatment	Conc. (%)	Mortality (%) [a] ± SD (min)				LT_{50} (min) (LCL-UCL)
		1	10	30	60	
C. aurantium EO	1	0f	0f	4.0 ± 1.0g	12.0 ± 2.4g	315.9 (261.4-392.4)
	5	60.0 ± 12.7c	60.0 ± 12.7c	60.0 ± 12.7c	60.0 ± 12.7c	15.0 (12.1-21.0)
d-limonene	1	88.0 ± 9.8b	100a	100a	100a	0.3 (0.1-0.7)
	5	100a	100a	100a	100a	0.2 (0.1-0.5)
E. globulus EO	1	30.0 ± 15.0de	30.0 ± 15.0d	30.0 ± 15.0e	36.0 ± 15.0e	154.3 (115.1-250.8)
	5	36.0 ± 14.9d	36.0 ± 15.0d	36.0 ± 15.0d	48.0 ± 9.8d	96.9 (63.6-140.5)
1,8-cineole	1	84.0 ± 2.0b	88.0 ± 16.0b	96.0 ± 8.0a	96.0 ± 8.0b	8.9 (-)
	5	100a	100a	100a	100a	0.2 (0.1-0.5)
benzyl benzoate (positive control)	25% (*w/v*)	16.0 ± 1.6e	16.0 ± 1.6e	20.0 ± 14.1f	28.0 ± 11.0f	223.8 (-)
ethyl alcohol (negative control)	70% (*v/v*)	0f	0f	0h	0h	n/a

[a] One-way ANOVA and Duncan's multiple range test results show that the means in each row that is followed by a different letter differ considerably ($P<0.05$).
LT_{50} = 50% lethal time; *UCL* is upper confidence limit; *LCL* is lower confidence limit.
n/a = not available.

Table 3. Mortality rate and LT_{50} of essential oils from *C. aurantium* and *E. globulus* and their major constituents against adults of *P. humanus capitis*, for an immersion time of 1 min

Treatment	Conc. (%)	Mortality (%) [a] ± SD (min)				LT_{50} (min) (LCL-UCL)
		1	10	30	60	
C. aurantium EO	1	28.0±16.0f	28.0±16.0f	28.0±16.0e	36.0±15.0e	94.2 (10.2-413.4)
	5	60.0±17.9d	60.0±17.9e	60.0±17.9c	64.0±15.0c	77.3 (18.7-143.7)
d-limonene	1	48.0±30.5e	80.0±17.9d	88.0±9.8b	96.0±8.0b	2.1 (0.5-11.1)
	5	96.0±8.0b	96.0±8.0b	100a	100a	1.2 (0.2-7.1)
E. globulus EO	1	12.0±9.8	12.0±9.8g	12.0±9.8f	12.0±9.8	314.5 (190.8-510.2)
	5	60.0±21.9d	60.0±21.9e	60.0±21.9c	60.0±21.9d	199.5 (51.0-654.5)
1,8-cineole	1	76.0±23.3c	88.0±16.0c	100a	100a	1.6 (1.0-7.9)
	5	100a	100a	100a	100a	0.2 (0.5-1.0)
Benzyl benzoate (positive control)	25% (*w/v*)	0g	0h	36.0±16.7d	36.0±16.7e	35.0 (-)
Ethyl alcohol (negative control)	70% (*v/v*)	0g	0h	0g	0f	n/a

[a] One-way ANOVA and Duncan's multiple range test results show that the means in each row that is followed by a different letter differ considerably ($P<0.05$).
LT_{50} = 50% lethal time; *UCL* is upper confidence limit; *LCL* is lower confidence limit.
n/a = not available.

Discussion

All tested EO and EO constituent formulations exhibited the highest insecticidal activity against nymphs and adults of *P. humanus capitis*. Especially, 5% 1,8-cineole exhibited the strongest insecticidal activity against nymphs and adults *P. humanus capitis* in terms of LT_{50} and LC_{50}. This conclusion was supported by Yang *et al*. (2004). They reported that 1,8-cineole at 0.25 mg/cm^2 had a strong insecticidal activity against female adults of *P. humanus capitis* with an LT_{50} of 14.4 min. A paper by Toloza *et al*. (2010) indicated that the KT_{50} of 1,8-cineole against *P. humanus capitis* adults was 11.10 min, and a paper by Yang *et al*. (2004) reported a strong insecticidal effect of *E. globules* EO, of which 1,8-cineole was the major constituent, against the adults of *P. humanus capitis* with LT_{50} of 4.2 min. In addition, 1,8-cineole is highly toxic to *M. domestica* larvae (Kumar *et al*., 2014). A major constituent of *E. globulus* EO was 1,8-cineole (44.54%), identified by GC-MS analysis (Soonwera and Sittichok, 2020). The mode of action of 1,8-cineole against insect pests was inhibition of their respiratory and digestive systems.

All formulations evaluated in this investigation displayed greater insecticidal efficacy against *P. humanus capitis* nymphs and adults than did 25% (*w/v*) benzyl benzoate. These findings agree well with those from Inthaphalan *et al*. (2022). Their paper concluded that a shampoo from extract of *Morinda citrifolia* leaf mixed with *Alpinia galanga* rhizome exhibited higher toxicity than that of 25% (w/v) benzyl benzoate. Recently, a researcher has reported the toxic effect of benzyl benzoate on humans. This synthetic pediculicide is harmful to humans. It causes severe skin irritation with an acute dermal LD_{50} of 4,000 mg/kg for rabbits (Johnson *et al*., 2017). The 1,8-cineole from *E. globulus* EO has been used successfully as a traditional antibacterial medicine in Asian nations since ancient times, in contrast to the toxic benzyl benzoate (Barbosa *et al*., 2016). It is non-toxic to humans and mammals, non-persistence, and quickly degraded in the environment (Pavela and Benelli, 2016; Soonwera and Sittichok, 2020). It has an acute dermal LD_{50} of 1500 mg/kg for mice and does not cause any abnormal skin reaction (Berhan *et al*., 2020). The results demonstrated the full potential of 1,8-cineole from *E. globulus* EO. It should be further developed into a pediculicidal shampoo product that is completely shown to be safe for humans, especially schoolchildren.

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