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Adulticidal effect of combinations between *Cymbopogon citratus* and *Eucalyptus globulus* essential oils against *Pediculus humanus capitis* De Geer

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Abstract The results showed that all head lice adults that were immersed in a combination of 15% *C. citratus* EO + 15% *E. globulus* EO, 20% *C. citratus* EO + 20% *E. globulus* EO, and 30% *C. citratus* EO + 30% *E. globulus* EO for 3 min were destroyed with the highest mortality rate of 100% at 30 min and an LT_{50} ranging from 1.2 to 1.5 min. However, 0.5% (*w/w*) permethrin shampoo provided a slightly higher adulticidal activity than all EO formulations in terms of LT_{50} (0.9 min). According to the LD_{50} values reported in the literature, *C. citratus* EO + *E. globulus* EO can be developed into a safer shampoo for head lice treatment.

Keywords: *Pediculus humanus capitis*, *Eucalyptus globulus* EO, *Cymbopogon citratus* EO, Adulticidal effect, Head louse infestation, Pediculicide

Introduction

Head louse infestation is the most common human parasitic infestations and is a worldwide public health concern including Thailand, particularly in the paediatric school-aged group. It is caused by *Pediculus humanus* capitis De Geer (Sittichok *et al*., 2018; Leung *et al*., 2021; Mohammadi *et al*., 2022). There are three stages in this insect's life cycle: the egg, the nymph, and the adult. It frequently appears in the hair above, behind, and next to the neck. Male and female adults and nymphs both consume human blood multiple times per day (Arserim *et al*., 2021; Soonwera *et al*., 2018).

Chemical-based pediculicides such as carbaryl (carbamate), permethrin, ivermectin, malathion (organophosphate), and dimethicone in various formulations (e.g., shampoo and lotion) have been applied for treatment and eradication of head lice infestation. Unfortunately, these chemical pediculicides are extremely hazardous to both humans and the environment and can only kill adults and

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nymphs—not eggs (Leung *et al*., 2021; Soonwera *et al*., 2018). Furthermore, it has been well known that head lice populations develop resistance to pediculicides. Permethrin exhibited a higher level of resistance with a resistance ratio (RRs) ranging from 162.5 to 655.2 folds (Vassena *et al*., 2003). Hence, there is an urgent need to develop safe and high efficacy alternatives for head lice control.

Plant-derived essential oils (EOs) exert adulticidal activity against head lice and other insect pests (Candy *et al*., 2020), in particular, EOs from *Cinnamomum cassia*, *Curcuma xanthorrhiza*, *Curcuma zedoaria*, *Eucalyptus globulus*, *Lippia multiflora*, *Mentha piperata*, *Origanum onites*, and *Zingiber zerumbet* (Arserim *et al*., 2021; Candy *et al*., 2020; Soonwera *et al*., 2018). In addition, several EOs have long been used as common food and folk medicine (e.g., anti-oxidant, anti-microbial, and anti-fungal) (Barbosa *et al*., 2016; Ranitha *et al*., 2014).

Insecticidal activities of mixtures of EOs and EO constituents have been investigated by several researchers. Choi *et al*. (2010) coucluded that a combination of *Eugenia caryophyllata* EO + *E*. *globulus* EO provided a strong synergistic adulticidal activity against female *P*. *humanus capitis*. Gallardo *et al*. (2012) stated that a combonation of EO constituents from geraniol + citronellol + citronellyl showed a strong synergistic adulticidal activity against *P*. *humanus capitis*. Audino *et al*. (2007) reported that lotions based on a combination of 5% peppermint + 5% eucalyptus in ethanol 96% provided a high synergistic insecticidal activity against *P*. *humanus capitis* with 93% knockdown rate. Soonwera and Sittichok (2020) reported that 5% *Cymbopogon citratus* EO + 5% *E*. *globulus* EO was synergistic. The combination provided a synergistic insecticidal activity against *Aedes aegypti*, *Aedes albopictus*, and *Musca domestica*.

The purpose of this study was to evaluate the effectiveness of combining *Cymbopogon citratus* and *Eucalyptus globulus* essential oils against adults of *Pediculus humanus capitis* De Geer.

Materials and methods

Ethical statement concerning head lice collect

The methodology for collecting head lice from humans (permission number 76-2558) has been accepted by the human ethics committee of the Institute for Development of Human Research Protection (IHRP). Bangkok, Thailand is where IHRP is located.

Sampling of head lice

Adults of head lice (females) were collected by a combing method (Sittichok *et al*., 2018) in the hair of 100 infested children (8-12 years old) at a primary school in Chachoengsao Province, Thailand, in October 2018. With the consent of the children's guardians, the youngsters were not previously treated with an anti-lice product for at least 1 month. After the head lice were carefully removed from the teeth of the lice comb, all 200 adult lice, used in all experiments, were placed in clean boxes (18.0 × 23.0 × 5.5 cm). The lice were kept under room conditions at 27±4 ℃ and 74±4% RH until the experiment started.

Plant materials and essential oil extraction method

Fresh *C. citratus* stems and fresh *E. globulus* leaves were procured in May and June 2018 from a farm in Thailand's northeastern Nakhon Ratchasima region (14°37' 9" N / 101°26'53" E). They were identified by a plant taxonomist from the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. One kilogram of fresh stems and leaves were cleaned, cut into small pieces, and extracted for EOs by a distillation method. The mixture was distilled for 5 h. Then, the EOs were extracted from the separating funnel, preserved in airtight containers, and refrigerated at 4 ℃ until use. For adulticidal activity assay, the combined EOs in ethyl alcohol formulations were prepared: 5% *C. citratus* EO + 5% *E. globulus* EO, 10% *C. citratus* EO + 10% *E. globulus* EO, 15% *C. citratus* EO + 15% *E. globulus* EO, 20% *C. citratus* EO + 20% *E. globulus* EO, and 30% *C. citratus* EO + 30% *E. globulus* EO. All formulations were prepared and stored under room conditions at 27±2 ℃ and 74±2% RH.

Toxicity assay against P. humanus capitis

The aduticidal activity assay in this study was an immersion test (Soonwera *et al*., 2018). The test was started within 30 min after the head lice were collected. Ten motile adult head lice were subjected to each treatment in the test. They were examined under a stereomicroscope (Nikon® Type 102) to confirm no gut and antennae movement, hence indicating mortality. In a 5-cm diameter petri dish with 1,000 µL of each EO formulation, ten head louse adults were immersed for 1 and 3 min. After the period of exposure, the head lice were dried on a Whatman® No. 1 filter paper (3 × 4 cm). At 1, 5, 15, 30, and 60

min, the head lice mortality on the filter paper was observed and recorded under a stereomicroscope. The strict criterion of head lice mortality was the total absence of any vital signs, including stomach movement, antennal movement, and leg movement stimulated or unstimulated by forceps (Sittichok *et al*., 2018). The experiment was performed using ten replicates for each treatment.

Permethrin shampoo, the positive control, was obtained from Sherwood Chemicals Co. Ltd. in Bangkok, Thailand (Scully Anti-Lice shampoo®, 0.5% (*w/w*) permethrin). The negative control, 70% (*v/v*) ethyl alcohol in water, was obtained from T.S. Interlab Limited Partnership in Bangkok, Thailand.

Statistical analysis

A completely randomized design was used for the experiments. LT_{50} (50% Lethal Time) value was calculated using probit analysis. Mortality data were statistically analyzed by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at $P<0.05$. All statistical analyses were done with SPSS Statistical Software Package version 23.0.

Results

The adulticidal activity, for 1-min immersion time, of every combination of EOs against *P. humanus capitis* is shown in Table 1. Among combinations of EO formulations, two combinations of 20% *C. citratus* EO + 20% *E. globulus* EO, and 30% *C. citratus* EO + 30% *E. globulus* EO provided stronger adulticidal activity against *P. humanus capitis* than the other formulations. The highest mortality rate was 100% at 60 min after exposure, and a short LT_{50} of 4.0 min was achieved by a combination of 30% *C. citratus* EO + 30% *E. globulus* EO, while the lowest mortality rate was 98.0%, at 60 min after exposure and a longer LT_{50} of 5.9 min, provided by a combination of 5% *C. citratus* EO + 5% *E. globulus* EO. Other formulations had LT_{50} between 4.7 to 5.9 min and mortality rate between 0.0-98.0%. In terms of LT_{50} value, the combination of 30% *C. citratus* EO + 30% *E. globulus* EO was more toxic to the adults of *P. humanus capitis* than the 0.5% (*w*/*w*) permethrin shampoo was (LT_{50} of 4.3 min) (positive control) (Figure 1). In contrast, 70% (*v/v*) ethyl alcohol (negative control) did not destroy any head lice adults at all.

The mortality rates, for 3-min immersion time, of every combination of EOs against *P. humanus capitis* are summarized in Table 2. Three combinations—15% *C. citratus* EO + 15% *E. globulus* EO, 20% *C. citratus* EO

+ 20% *E. globulus* EO, and 30% *C. citratus* EO + 30% *E. globulus* EO—exerted a stronger adulticidal activity against *P. humanus capitis* than the other two formulations. They also showed the highest mortality rate (100% at 30 min after exposure), while the two formulations—5% *C. citratus* EO + 5% *E. globulus* EO, 10% *C. citratus* EO + 10% *E. globulus* EO—showed a mortality rate ranging from 94.0-98.0%. A combination of 30% *C. citratus* EO + 30% *E. globulus* EO had the strongest adulticidal activity against adults *P. humanus capitis* with an LT_{50} of 1.2 min, whereas other formulations showed an LT_{50} ranging from 1.4 to 2.1 min. In contrast, 0.5% (*w/w*) permethrin shampoo was more effective than all tested formulations of combined EOs, in terms of LT_{50} value (Figure 1), while 70% (*v/v*) ethyl alcohol (negative control) did not destroy any head lice adults at all.

Table 1. Effects of formulations of combined EOs on the mortality rate and LT_{50} value of *P. humanus capitis* for an immersion time of 1 min

Treatment	Mortality (%) [a] ± SD (min)					LT_{50} (min)
	1	5	15	30	60	(LCL-UCL)
5% *C. citratus* EO + 5% *E. globulus* EO	0.0c	40.0±3.7c	84.0±1.1c	94.0±0.9b	98.0±0.5b	5.9 (3.5-7.1)
10% *C. citratus* EO + 10% *E. globulus* EO	0.0c	32.0±3.1de	86.0±2.6c	92.0±1.3c	98.0±0.5b	5.4 (0.6-7.0)
15% *C. citratus* EO + 15% *E. globulus* EO	14.0±2.0b	64.0±1.5a	90.0±1.2b	94.0±0.9b	98.0±0.5b	4.8 (0.8-9.0)
20% *C. citratus* EO + 20% *E. globulus* EO	26.0±4.2a	52.0±1.1b	88.0±0.8bc	94.0±0.9b	100a	4.7 (2.6-6.4)
30% *C. citratus* EO + 30% *E. globulus* EO	0.0c	36.0±3.6d	76.0±1.7d	92.0±1.3c	100a	4.0 (2.16-4.7)
0.5% (*w/w*) permethrin shampoo (positive control)	0.0c	30.0±4.1e	98.0±0.5a	100a	100a	4.3 (1.2-5.3)
70% (*v/v*) ethyl alcohol (negative control)	0.0c	0.0f	0.0d	0.0d	0.0c	n/a

[a] The means in each row that are denoted by a different letter differ considerably (*P*<0.05, according to Duncan's Multiple Range Test and one-way ANOVA).
LT_{50} = 50% lethal time; *LC_{50}* = 50% lethal concentration; *UCL* is upper confidence limit; *LCL* is lower confidence limit. n/a = not available.

Table 2. Effects of formulations of combined EOs on the mortality rate and LT_{50} of *P. humanus capitis*, for an immersion time of 3 min

Treatment	Mortality (%) [a] ± SD (min)					LT_{50} (min)
	1	5	15	30	60	(LCL-UCL)
5% *C. citratus* EO + 5% *E. globulus* EO	52.0 ±1.1b	62.0 ±1.3bc	82.0 ±0.8c	94.0 ±0.9c	98.0 ±0.5c	2.1 (0.4-4.1)
10% *C. citratus* EO + 10% *E. globulus* EO	36.0 ±2.2d	72.0 ±1.3b	98.0 ±0.5b	98.0 ±0.5b	100a	2.0 (0.8-8.9)
15% *C. citratus* EO + 15% *E. globulus* EO	38.0 ±1.5d	60.0 ±1.3c	94.0 ±0.6b	100a	100a	1.5 (0.1-2.2)
20% *C. citratus* EO + 20% *E. globulus* EO	48.0 ±1.3g	60.0 ±1.3c	82.0 ±0.8c	100a	100a	1.4 (0.6-4.2)
30% *C. citratus* EO + 30% *E. globulus* EO	12.0 ±0.8e	60.0 ±1.3c	100a	100a	100a	1.2 (0.8-2.7)
0.5% (*w/w*) permethrin shampoo (positive control)	61.0 ±1.7a	100a	100a	100a	100a	0.9 (0.8-2.9)
70% (*v/v*) ethyl alcohol (negative control)	0.0f	0.0d	0.0d	0.0d	0.0d	n/a

[a] The means in each row that are denoted by a different letter differ considerably *(P*<0.05, according to Duncan's Multiple Range Test and one-way ANOVA).
LT_{50} = 50% lethal time; LC_{50} = 50% lethal concentration; *UCL* is upper confidence limit; *LCL* is lower confidence limit.
n/a = not available.

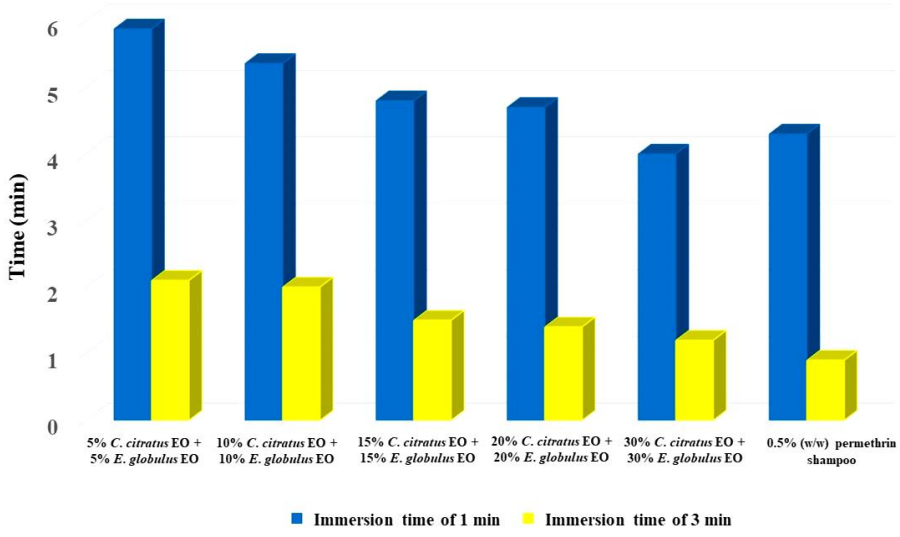

Figure 1. 50% Lethal Time (LT_{50}) of formulations of combined EOs against adults *P. humanus capitis* for an immersion time of 1 and 3 min

Discussion

In this study, for 3 min immersion time, three formulations—15% *C. citratus* EO + 15% *E. globulus* EO, 20% *C. citratus* EO + 20% *E. globulus* EO, and 30% *C. citratus* EO + 30% *E. globulus* EO—provided the strongest adulticidal activity against *P. humanus capitis* with 100% mortality rate at 30 min and an LT_{50} ranging from 1.2 to 1.5 min. The outcomes of this study are consistent with those of other research. Soonwera and Sittichok (2020) indicated that against three medical and veterinary insects, 5% *C. citratus* EO + 5% *E. globulus* EO showed a significant insecticidal effect with 100% mortality rate and showed a KT_{50} of 1.4 min against *Ae. aegypti*, 1.8 min against *Ae. albopictus*, and 3.4 min against *M. domestica*. Choi *et al*. (2010) reported that a combination of *Eugenia caryophyllata* EO + *E. globulus* EO (3:7 ratio) at 20 mL was highly toxic against female *P. humanus capitis* with 98% mortality rate. Chauhan *et al*. (2016) indicated a strong insecticidal effect of two combinations of EOs—*Mentha piperita* EO + *C. citratus* EO and *M. piperita* EO + *E. globulus* EO (50:50 ratio)—against second-instar larvae of housefly (*M. domestica*) with an LC_{50} ranging from 1.07-2.25 (μl/cm^2). Toloza *et al*. (2008) discovered that a combination of *E. grandis* EO + *E. tereticornis* EO had a potent insecticidal impact on *P. humanus capitis*.

On modes of action of these EOs against *P. humanus capitis*. Aungtikun *et al*. (2021) and Soonwera and Sittichok (2020) reported that geranial from *C. citratus* EO and 1,8-cineole from *E. globulus* EO were the cause of mortality of many insect pests including head lice. They worked by inhibiting neural cells and acetylcholinesterase (AChE) enzymes of the neuroreceptors.

In contrast, the LT_{50} of 0.5% (*w/w*) permethrin shampoo (positive control), was 0.9 min, which was longer than the LT_{50} of all tested formulations of combined EOs for 3-min immersion time, except a combination of 30% *C. citratus* EO + 30% *E. globulus* EO. For a 3-min immersion time, the formulation of combined EOs showed a shorter lethal time (LT_{50}) than 0.5% (*w/w*) permethrin shampoo did. This finding was supported by a study by Sittichok *et al*. (2018), showing that 0.5% (*w/w*) permethrin pediculicide at 0.05 mL/cm^2 exhibited the most effective insecticidal activity against adults of *P. humanus capitis* (28.0% mortality rate) than a shampoo of combined *Moringa oleifera* EO + eucalyptus EO (20.0% mortality rate). This study found that permethrin shampoo was more toxic than the tested EOs, in contrast to several other studies that reported that due to insect resistance to chemical pediculocides, permethrin was less toxic than several tested EOs. For example, Yingklang *et al*. (2022) found that *Callistemon viminalis* EO was more toxic to the adults of *P. humanus capitis* than 1% (*w/w*) permethrin. Other examples are

papers reported by Leung *et al*., (2021) and Grieve *et al*., (2007). Unfortunately, permethrin is a neurotoxic insecticide that affects the nervous system of humans, with an acute oral exposure LD_{50} ranging from 2280–3580 mg/kg for humans and an acute dermal LD_{50} of >2500 mg kg^{-1} (rat) and >2000 mg kg^{-1} (rabbit), and head lice resistance to it has already been developed (Leung *et al*., 2021). It causes severe side effects after treatment such as pruritus, burning and erythema (Chrustek *et al*., 2018; Sittichok *et al*., 2018). Meanwhile, *C*. *citratus* EO and *E*. *globulus* EO are much less dangerous than permethrin and swiftly decompose in the environment. They are also non-bioaccumulating. (Soonwera and Sittichok, 2020). Furthermore, they are non-toxic to humans and mammals, with an oral LD_{50} of 3811.5 mg/kg^{-1} for male rats (*E*. *globulus* EO) and 7046 mg/kg^{-1} for mice (*C*. *citratus* EO) (Soonwera and Sittichok, 2020). Moreover, they do not cause any abnormal skin reaction in rat and rabbit (Lulekal *et al*., 2019; Berhan *et al*., 2020).

Therefore, a combination of *C*. *citratus* EO + *E*. *globulus* EO had a high potential to be developed into a green pediculicide in shampoo formulation for head lice control because it is safer to children.

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