

Ovicidal and Repellent Activities of Several Plant Essential Oils Against *Periplaneta americana* L. and Enhanced Activities from their Combined Formulation

Mayura Soonwera (✉ mayura.so@kmitl.ac.th)

King Mongkut's Institute of Technology Ladkrabang

Tanapoom Mounghipmalai

King Mongkut's Institute of Technology Ladkrabang

Wacharaporn Takawirapat

King Mongkut's Institute of Technology Ladkrabang

Sirawut Sittichok

King Mongkut's Institute of Technology Ladkrabang

Research Article

Keywords: Ovicidal, Repellent Activities, Plant Essential Oils, Combined Formulation

Posted Date: December 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1091267/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Natural ovicidal and repellent agents against *Periplaneta americana* are in urgent need, and plant essential oils (EOs) can assume this role quite readily. In this study, ovicidal and repellent activities against *Periplaneta americana* of EOs from *Cymbopogon citratus*, *Cinnamomum verum*, *Eucalyptus globulus*, *Illicium verum*, and *Zanthoxylum limonella* in soybean oil and in ethyl alcohol were determined by topical and dual-choice assays, as well as 10% cypermethrin and a combined formulation of 5% *C. verum* EO + 5% *I. verum* EO. Cypermethrin at 10% provided the highest toxicity (100% inhibition rate) against the eggs, but only slightly higher than that (99.3%) provided by the combined EO formulation, while the highest repellent activity against the adults was provided by the combined formulation (89.5% repelled cockroaches at 48 h after treatment). In addition, all EO formulations in soybean oil provided higher ovicidal and repellent activities than in ethyl alcohol. To conclude, the combined EO formulation in soybean oil can replace cypermethrin because their efficacy was nearly equivalent, but the combination should be much safer to use.

Introduction

Cockroaches are unhygienic scavenger insect pest in human settlements¹. They belong to the Order Blattodea². Only 25-30 out of 4,500-4,600 cockroach species are associated with human habitation and classified as pests²⁻³. American cockroach, *Periplaneta americana* L. (*P. americana*) (Blattidae), is one of the most serious pests in houses, buildings, farms, and livestock areas³⁻⁴. The color of this insect pest can be reddish brown to brownish black, with a body length of 34-53 mm. Their body shape is a flattened oval shape. The body of a male adult is longer than that of a female adult³⁻⁴. Female adults deposit one ootheca (egg case) per month for at most ten months. Each ootheca contains 14-16 eggs³⁻⁴. *P. americana* undergoes incomplete metamorphosis in its life cycle. Its three life stages of egg, nymph, and adult can extend from 168 to more than 700 days³⁻⁴. Eggs may take between 30 to 60 days to hatch into nymphs. Nymphs grow into adults after 6 to 12 months. The adult life span was 1-12 months, and a female cockroach can reproduce a total of 100-150 young nymphs during her lifetime³⁻⁴. The length of each of its life stages as well as the length of its lifetime are influenced by temperature, humidity, and amount of available food³⁻⁴. It is a nuisance pest that causes psychological distress and public health problems because it mechanically transmits over 100 types of human pathogenic organisms, food-borne pathogens, and parasites, such as *Citrobacter freundii*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Staphylococcus aureus*, *S. epidermidis*, *Aspergillus niger*, *Saccharomyces cerevisiae*, *Ascaris lumbricoides*, *Balantidium coli*, *Enterobius vermicularis*, *Entamoeba histolytica*, *Schistosoma haematobium*, and *Trichuris trichiura*⁵⁻⁸. Today, *P. americana* is everywhere around the globe, spread throughout it by global commerce⁵⁻⁶. Long-term control of *P. americana* is complicated because it is highly adaptable. It can feed not only on all human foods but also many other organic objects that are not human foods (book binding, cardboard, fresh & dried blood, excrement, sputum, baby fingernail, dead & crippled cockroaches)³⁻⁴. At the time of the study, it is resistant to most conventional insecticides, such as malathion, carbaryl, permethrin, and cypermethrin⁹⁻¹⁰. An accepted strategy for current cockroach control is to reduce the use of synthetic insecticides¹¹. The ultimate goal for this research project was to find a safer and at least as effective alternative to these synthetic insecticides because they (e.g., organophosphates and carbamates) are deadly poisonous to humans¹²⁻¹³.

Many researchers suggested using plant extracts and plant essential oils (EOs) as good alternatives¹⁴⁻¹⁷ to synthetic insecticides. There have been a large number of reports that some EOs exhibited strong repellent activity against *P. americana* adults: *Boesenbergia rotunda*, *Citrus hysteric*, *Cymbopogon citratus*, *C. nardus*, *Curcuma longa*, *Eucalyptus citriodora*, *Jatropha curcas*, *Litsea cubeba*, *Mentha arvensis*, *Piper nigrum*, *Psidium guajava*, *Syzygium aromaticum*, and *Zingiber officinale*¹⁸⁻²¹. In addition, a combination of *E. globulus* + *Rosmarinus officinalis* EOs showed a stronger insecticidal effect against adults *P. americana* than that of each individual EO¹⁶. Finally, EOs are much safer and more environmentally friendly than conventional synthetic insecticides^{10,14}.

Therefore, the first objective of this work was to determine the ovicidal and repellent activities of EOs extracted from the following plant parts: *C. citratus* stems, *Cinnamomum verum* (*C. verum*) barks, *Eucalyptus globulus* (*E. globulus*) leaves, *Illicium verum* (*I. verum*) fruits, and *Zanthoxylum limonella* (*Z. limonella*) fruits. The second objective was to combine the EO that provided the highest ovicidal activity with the EO that provided the highest repellent activity into a strong formulation against the eggs and adults of american cockroach, *P. americana* and determine the formulation's potency. All EOs presented in this study have been reported before to possess an insecticidal activity as well as be safe for humans²²⁻²⁶. EOs have been used safely since ancient time as folk medicine—they can destroy some human pathogenic organisms²²⁻²³. Furthermore, *C. citratus*, *C. verum*, *I. verum*, and

Z. limonella have been long used as spices in Thai and Asian foods^{24, 27-29}. If the combined formulation was demonstrated to be equivalently effective to cypermethrin, it can be developed into a safer ovicidal and repellent agent for comprehensive control of cockroach populations in epidemic areas, which can help eliminate human pathogenic organisms transmitted by *P. americana*.

Results

Essential oils (EOs) of *C. citratus* stems, *C. verum* barks, *E. globulus* leaves, *I. verum* fruits, and *Z. limonella* fruits were extracted by a water distillation method, and their chemical constituents were identified by GC-MS. The identified constituents and the percentage yield of each EO are listed in Table 1. EO with the highest percentage extraction yield was *Z. limonella* EO (9.27% v/w), followed by *I. verum* EO (3.23% v/w), *E. globulus* EO (1.21% v/w), *C. citratus* EO (1.15% v/w), and *C. verum* EO (1.10% v/w). The *C. citratus* EO was composed of 10 chemical constituents, accounting for 97.40% of the chemical profile. Its major constituents were geranial (46.33%), neral (25.72%), and 1-8-cineole (10.93%). The *C. verum* EO was composed of 11 chemical constituents, accounting for 98.60% of the chemical profile. Its major constituents were cinnamaldehyde (75.23%) and benzene (14.15%). As for the *E. globulus* EO, it was composed of 19 chemical constituents, accounting for 97.35% of the chemical profile. Its major constituents were 1,8-cineole (45.82%) and γ -terpinene (20.13%). The *I. verum* EO was composed of 10 chemical constituents, accounting for 98.58% of the chemical profile. Its major constituent was trans-anethole (92.24%). As for *Z. limonella* EO, it was composed of 16 chemical constituents, accounting for 97.75% of the chemical profile. Its major constituents were limonene (28.13%) and terpinen-4-ol (24.13%).

The inhibition rates of every treatment and control against *P. americana* eggs at 30 days after exposure are tabulated in Table 2. All treatments and control were 10% solutions of a compound in soybean oil and in ethyl alcohol. The inhibition rate of every EO formulation in soybean oil (85.3-96.7%) was higher than that of the same EO in ethyl alcohol (60.3-90.1%). The highest egg inhibition rate, at 96.9%, among all 10% EO solutions in soybean oil, was exhibited by *I. verum* EO, and the lowest egg inhibition rate, at 85.3%, was shown by *C. verum* EO. The highest inhibition rate among all 10% EO formulations in ethyl alcohol, at 90.1%, was achieved by *Z. limonella* EO, and the lowest, at 60.3%, was exhibited by *C. citratus* EO. The implications of these highest and lowest inhibition rate results are separately discussed in the discussion section. In contrast, 10% w/v cypermethrin both in soybean oil and in ethyl alcohol exhibited a full 100% inhibition rate, the highest among all treatments. Regarding inhibition rate index (IRI), every EO formulation showed an IRI of less than 1 (0.6-0.97), i.e., they were less toxic against *P. americana* eggs than 10% w/v cypermethrin.

The percentage repellent rates against *P. americana* adults at 48 h of exposure of treatments and control in soybean oil and in ethyl alcohol are shown in Table 3. All 10% solutions in soybean oil of the five plant EOs exhibited a high percentage range of repellent activity, of 60.8-100%, against *P. americana* adults, and each of them exhibited a higher percentage repellent activity than the same EO in ethyl alcohol (47.6-88.2%).

There was an inverse relationship between percentage repellent activity and time of exposure. As the time of exposure increased, the repellent activity percentage decreased, as can be seen as a regression curve in (Fig. 2). This kind of relationship existed for all tested EO formulations, both in soybean oil and in ethyl alcohol. For example, soybean oil solution of *C. verum* showed the highest percentage repellent activity of 100% after 1 h of exposure, while after 48 h of exposure, it showed a lower 83.4%. For another example, *C. citratus* soybean oil solution showed 98.0% repellent activity after 1 h of exposure, but 79.0% after 48 h of exposure. Following the same trend, after 1 h of exposure, ethyl alcohol solution of *C. verum* showed 84.8% repellent activity, while after 48 h of exposure, it showed a lower 62.8%. Similarly, ethyl alcohol solution of *C. citratus* showed 88.2% repellent activity after 1 h of exposure, while it showed a lower 62.0% after 48 h of exposure.

Both 10% cypermethrin solutions in soybean oil and in ethyl alcohol showed a lower percentage repellent activity against *P. americana* adults than every EO solution in both kinds of solvent, at any length of exposure time. Cypermethrin solution in soybean oil (73.4% repellent rate) was only slightly more potent than cypermethrin in ethyl alcohol (69.6% repellent rate) after 1 h of exposure.

The effective repellent index (ERI) against *P. americana* adults of each of 10% solutions of five plant EOs and cypermethrin in soybean oil and in ethyl alcohol are presented in Table 4. Indicated by the ERI, all EO formulations were more repellent against *P. americana* adults than 10% cypermethrin. The ERIs of all EO formulations were always higher than that of cypermethrin at any time of exposure. The highest ERI observed was 1.71, achieved by 10% *C. verum* EO in soybean oil after 48 h of exposure, signifying that this EO formulation was 1.7 times more potent than 10% cypermethrin. Other EO formulations showed an ERI in the range of 1.09-1.62.

As can be observed in Table 5 and Table 2, the ovicidal inhibition rate exhibited by the combined formulation was considerably higher (99.3%) than that exhibited by 10% *I. verum* EO (91.2%). In the same vein, the repellent activity against *P. americana* adults after 48 h of exposure exhibited by the combined formulation of 5% *I. verum* EO + 5% *C. verum* EO (see Table 6) was higher (89.5%) than that exhibited by *C. verum* EO (83.4%) (Table 3). Moreover, both in ethyl alcohol and soybean oil solvents, formulation of 5% *I. verum* EO + 5% *C. verum* EO combination exhibited a range of inhibition rates, 91.9-99.3%, that was very close to the 100% inhibition rate of cypermethrin. The most significant result was that the IRI of the combined EO formulation in soybean oil was very close to one (i.e., 0.99), indicating that its potency was almost identical to that of cypermethrin. Finally, the combined formulation in soybean oil (99.3% ovicidal inhibition rate) exhibited a higher efficacy than the combined formulation in ethyl alcohol (91.9% inhibition rate).

As can be observed in Table 6 and Table 3, the percentage repellent activity exhibited by the combined formulation was considerable higher than that exhibited by 10% *C. verum* EO and 10% cypermethrin. The combined formulation of 5% *I. verum* EO + 5% *C. verum* EO exhibited the highest repellent activity of 89.5% at 48 h exposure, while 10% cypermethrin showed 44.7% repellent activity. The most significant result of this study was that the ERI of this combined EO formulation in both soybean oil and ethyl alcohol solvent was in the range of 1.47-1.94, signifying that this combined formulation was 1.47-1.94 times more potent in repellent activity than 10% cypermethrin. Regarding the influence of solvent on efficacy, following the same trend of influence on ovicidal inhibition rate mentioned above, the repellent activity exhibited by the combined formulation in soybean oil (89.5%) was higher than the activity exhibited by the combined formulation in ethyl alcohol (65.7%).

Discussion

Several factors affected the extraction yield of plant essential oil, such as agricultural practice, cultivation area, and extraction method²⁶. The observed essential oil yields from the five plants in this study were checked and confirmed against the values reported in the literature, and mostly, they agreed very well. Namely, the essential oil yield of *C. citratus* observed in this study was 1.15% v/w, while papers by Aungtikun et al.²⁸, Soonwera and Sittichok²⁴, and Verma et al.³⁰ reported the yield of *C. citratus* as 0.50-1.5% v/w; the observed *C. verum* EO yield was 1.10% v/w, well-matched with 1.01-1.14% v/w reported by Aungtikun and Soonwera²⁷ and Li et al.³¹; the observed *E. globulus* EO yield was 1.21% v/w (water distillation extraction method), well-matched with 1.2-3.0 % w/w (water distillation) reported by Sebei et al.³²; and 1.10% v/w (steam distillation) reported by Soonwera and Phasomkusolsil²⁹ and Joshi et al.²⁵. The observed *I. verum* EO yield was 3.23% v/w, slightly lower than 4.0-4.5% v/w, (water distillation) reported by Gholivand et al.³³ and 4.0 % v/w, (water distillation) reported by Aungtikun et al.²⁸, and the observed yield of *Z. limonella* EO was 9.27% v/w, slightly lower than 9.63% v/w reported by Charoensup et al.²⁶. Moreover, it has been known that, although local species of *C. citratus* in Thailand and in India had the same number of constituents, the percentages of the constituents in their chemical profile were different³⁴. Therefore, those yield percentages were determined and checked against the values in recent literature. Many factors influenced the percentage of a major constituent in an EO's chemical profile, such as plant maturity, harvesting time, and good harvesting practice²⁸. The observed GC-MS chemical profiles of every EO were well-matched with those reported in a previous study. Namely, the percentage of geranial, the major chemical constituent of *C. citratus* EO observed in this study, was 46.33%, while papers by Aungtikun et al.²⁸, Soonwera and Sittichok²⁴, and Chauhan et al.³⁴ reported the percentage geranial at 42.40-49.69% of the EO chemical profile; in addition, the observed major constituent of *C. verum* EO was cinnamaldehyde at 75.23%, well-matched with 73.21% of the chemical profile reported by Aungtikun and Soonwera²⁷ and with 74.49% reported by Li et al.³¹, and lower than 90.17% reported by Chansang et al.³⁵; the observed major constituent of *E. globulus* EO was 1,8-cineole, at 45.82%, well-matched with 42.60-44.54% of the chemical profile reported by Soonwera and Sittichok²⁴ and Cotchakaew and Soonwera³⁶; the observed major constituent of *I. verum* EO was trans-anethole, at 92.24%, well-matched with 88.32-94.0% of the chemical profile reported by Aungtikun et al.²⁸ and Junior et al.³⁷; and the observed major constituent of *Z. limonella* EO was limonene, at 28.13%, slightly higher than 18.62% reported by Imphat and Woottisin³⁸, and lower than 43.63% reported by Charoensup et al.²⁶, and 57.94% reported by Wongkattiya et al.³⁹.

Nevertheless, there were some slight discrepancies, such as the yield and percentage in the chemical profile of the major constituents of *I. verum* and *Z. limonella* EOs. These discrepancies were likely due to usage of different plant parts for EO extraction, a difference in the extraction procedures, and variations in genotypic, phenotypic, and agro-ecological factors (e.g., plant age, growth phase in plant development, amount of sunlight and moisture, as well as the temperature at the farm's geographical location⁴⁰).

High toxicity of plant EO in soybean oil against insect pest has been reported in recent literature. A study on EO toxicity against head louse eggs by Soonwera et al.⁴¹ concluded that Zingiberaceae plant EOs in soybean oil exhibited high toxicity to the eggs of head louse (*Pediculus humanus capitis*; Pediculidae: Phthiraptera), with a full 100% inhibition rate. In this study, 10% *I. verum* EO and the combined EO formulation in soybean oil showed a high egg inhibition rate of 96.9% and 99.3%, respectively, against *P. americana*. This result had been expected before the experiment started because the outcomes of many studies on *I. verum* EO against eggs of insect pests other than *P. americana* have been reported in the literature. Specifically, Sinthusiri and Soonwera⁴² reported that 10% *I. verum* EO exhibited a markedly high oviposition deterrent and ovicidal activities against female houseflies (*Musca domestica*; Muscidae: Diptera), while Matos et al.⁴³ reported that *I. verum* EO exhibited a high inhibition rate against the eggs of cowpea weevil, *Callosobruchus maculatus* (Bruchidae: Coleoptera). Besides a high repellent activity against the adults, 10% *C. verum* EO and the combined formulation in soybean oil also exhibited a high repellent activity against *P. americana* adults—100% and 83.4% and 100% and 89.5% repellent activity at 1 and 48 h after exposure, respectively. Many papers reported a high repellent activity of *C. verum* EO against many insect pests such as *Plodia interpunctella* (Pyralidae: Lepidoptera), *Bemisia tabaci* (Aleyrodidae: Homoptera), *Ae. aegypti* (Culicidae: Diptera), *Sitophilus zeamais* (Curculionidae: Coleoptera)⁴⁴, *Sitophilus oryzae* (Curculionidae: Coleoptera)⁴⁵, and ant⁴⁶.

Unlike the large number of papers on individual EOs against *P. americana* mentioned above, papers on combined EO formulations against *P. americana* are limited. An example is a paper by Zibaee and Khorram¹⁶ reporting that a combined formulation of *E. globulus* + *R. officinalis* EOs exhibited a stronger insecticidal effect against adults *P. americana* than that of each individual EO component. Another one is by Aungtikun et al.²⁸ reporting that a combined formulation of 0.5% *I. verum* EO + 0.5% geranial showed a higher insecticidal effect against adult house flies, *M. domestica* than each individual EO.

Most notably, all EO formulations in soybean oil exhibited higher ovicidal and repellent activities against *P. americana* eggs and adults than all EO formulations in ethyl alcohol, in full agreement with findings from Sittichok and Soonwera⁴⁷ that eight herbal EOs in soybean oil showed a higher inhibition rate against *P. americana* eggs than the same EOs in ethyl alcohol. Findings on repellent activity against adult mosquitoes in studies by Soonwera and Phasomkusosil⁴⁸ and Phasomkusosil and Soonwera⁴⁹ showed the same trend: namely, 10% EOs from *C. citratus*, *C. nardus*, *Cananda odorata*, *Ocimum basilicum*, and *Z. cassumunar* in soybean oil were more repellent and feeding deterrent against adult females of *Anopheles minimus*, *Ae. aegypti*, and *Culex quinquefasciatus* than the same EO in ethyl alcohol. In the same vein, in this study, *C. verum* EO and the combined EO formulation exerted its potency better in a soybean oil than in ethyl alcohol.

The reason that a soybean oil solution of an EO is more repellent the same EO in ethyl alcohol solution is that soybean oil is more lipophilic than ethyl alcohol, and a lipophilic solvent retarded evaporation of EOs⁴⁹. In this study, *C. verum* EO and the combined EO formulation in soybean oil provided a longer, more persistent repellent activity against *P. americana* than the same EO or combined EOs in ethyl alcohol, and the higher activity was attributed to this reason. On the other hand, an EO in a hydrophilic solvent evaporated faster, hence shorter, less persistent repellent activity. This claim is supported by a paper by Phasomkusosil and Soonwera⁴⁹: 10% *Citrus sinensis* EO in soybean oil was more repellent (30-60 min) against adult females of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. minimus* than the EO in ethyl alcohol (less than 1.0 min). Another paper by Phasomkusosil and Soonwera⁴⁹ and Phukerd and Soowera⁵⁰ reported that 10% *Z. cassumunar* EO in soybean oil was more repellent (70 min) against adult females of *Ae. aegypti* than the EO in ethyl alcohol (30.0 min).

On the other hand, even though the reason for *I. verum* EO and the combined EO formulation were more potent in ovicidal activity in soybean oil than in ethyl alcohol is also the higher lipophilicity of soybean oil, the mechanisms of action were not the same. The mechanism responsible for the higher ovicidal activity is that a lipophilic solvent can carry an EO through insect cuticles more readily because insect cuticles are mainly lipophilic⁴¹, hence more EO passes into the insect, providing more ovicidal activity from a higher amount of EO in its body^{41,51-52}. This claim is supported by a conclusion from Rajashekar and Shivanandappa⁵³ and Jankowska et al.⁵⁴ that embryo and egg mortality were strongly affected by EO's high cuticle permeability. Outcomes of this experiment confirm the expectation that every EO in soybean oil was more effective than the same EO in ethyl alcohol.

Regarding the high ovicidal activity (96.9-99.3%) against *P. americana* eggs of 10% *I. verum* EO among all tested individual EO formulations, this high activity is likely to be provided by its main constituent, trans-anethole²⁸. Strong potency of trans-anethole against mosquito and housefly was reported by Aungtikun et al.²⁸ and Pavela⁵⁵. Its mechanism of action was reported in Jankowska

et al.⁵⁴ and Bosch-Serra et al.⁵⁶: namely, trans-anethole inhibited development of insects by blocking cytochrome P450 detoxification enzymes, retarding cell growth and juvenile hormone production as well as weakening the immune system, leading to eventual death.

In the same way, the high repellent activity (100% at 1 h) against *P. americana* adults of 10% *C. verum* EO is likely to be provided by its main constituent, cinnamaldehyde⁴⁷. Cinnamaldehyde has been reported to strongly repel *P. americana* adults (75.23%)⁴⁷. The repellent activity of cinnamaldehyde is strong because it causes serious damage to their respiratory system⁴⁷, so they always try to avoid it. This conclusion is supported by a claim by Devi and Devi⁵⁷ that the mechanisms of action of cinnamaldehyde against insect pest were inhibition of its respiratory system by enzyme inhibition, cell membrane alteration, reduced cell membrane integrity, and reduced cell respiration.

The overall findings from this study indicate that the combined formulation of 5% *C. verum* EO + 5% *I. verum* EO would be a good current alternative to cypermethrin because, firstly, it is equally or more potent than cypermethrin in ovicidal and repellent activities and, secondly, they should be less harmful to humans and non-target organisms as well as not be as persistent as cypermethrin in the environment. Findings supporting the first reason were the following. Firstly, against *P. americana* adults, *C. verum* EO and the combined EO formulation were 1.71-1.97 times more potent than cypermethrin in repellent activity, while the ovicidal activity of cypermethrin was only slightly more potent than those of *I. verum* EO and the combined EO formulation. Moreover, these findings are supported well by findings from a previous study by Sinthusiri and Soonwera⁴² that 10% cypermethrin showed a 100% inhibition rate against house fly eggs, while 10% *I. verum* EO showed a 97.33% rate. Secondly, findings by Ichikawa⁵⁸ and Sharma et al.⁵⁹ support the safety reason for replacing cypermethrin with a plant essential oil: cypermethrin is seriously toxic to the nervous and immune systems of humans, especially pregnant women and children, while findings by Aungtikun and Soonwera²⁷ and Patra et al.⁶⁰ support the claim that EOs are quickly degraded in the environment. Moreover, its safety to humans has been long established in Southeast Asia because it has been widely used as folk medicine since ancient time, and today, it is an irreplaceable food ingredient in some Thai menus as well as an active ingredient in modern, scientifically tested medicine (Tamiflu, an anti-influenza drug).

To conclude, findings in this study indicate that 10% EO of *I. verum* provided the highest ovicidal activity against *P. americana* eggs among the five tested EOs, while 10% *C. verum* provided the highest repellent activity against *P. americana* adults. Moreover, they suggest that a formulation of their combination (5% *I. verum* EO+ 5% *C. verum* EO) in soybean oil can replace cypermethrin as an equally potent but much safer alternative agent for controlling *P. americana* populations.

Materials And Methods

Plant materials

This study investigated five EOs extracted from some part of the following five plants: *C. citratus*, *C. verum*, *E. globulus*, *I. verum*, and *Z. limonella*. Fresh stems of *C. citratus* (KMITL-CC), and fresh leaves of *E. globulus* (KMITL-EG) were collected from an organic farm run by King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand (13.7563° N latitude, 100.5018° E longitude) and permit number KDS 2021/002, permitted by Dean of Faculty of Agricultural Technology, KMITL (Asst. Prof. Dr. Thongchai Phutthongsiri). Dried fruits of *Z. limonella* (KMITL-ZL) were purchased from a local market in Nan province, northern Thailand. Dried barks of *C. verum* (KMITL-CV) and dried fruits of *I. verum* (KMITL-IV) were purchased from a Chinese medicine shop in Bangkok. Scientific identification of five plants were accomplished by Dr. Sirawut Sittichok and Miss Jirapon Aungtikun, the herbal specialist at the KMITL Herbarium. All voucher specimen was deposited at the KMITL Herbarium, Faculty of Agricultural Technology, KMITL for future reference. This study complied with relevant local (KMITL) and national (the National Research Council of Thailand: NRCT) guidelines and legislation of Thailand. Photographs of those parts of *C. verum* and *I. verum* are displayed in Fig. 1, and the names of their major chemical constituents are listed in Table 1.

Extraction of plant essential oils

All tested plant EOs were extracted by the same hydro-distillation method²⁷⁻²⁸. In brief, 1,000 g of each plant part were cleaned, cut into small pieces, crushed, and extracted of its EO. The extraction was completed in 5-6 h. Each EO was collected in 50 ml brown airtight vials and preserved at 4°C for further bioassay and chemical constituent analysis. Each EO and a combined formulation of the EO with the highest ovicidal activity and the EO with the highest repellent activity was prepared into 10% solutions in soybean oil

and in ethyl alcohol. The soybean oil solvent was pure soybean oil, but the ethyl alcohol solvent was 70% (v/v) ethyl alcohol in water. All formulations were stored under laboratory conditions (27±2°C and 70±3% RH) until they were assayed.

Chemical constituent analysis

Chemical constituents of EOs from *C. citratus*, *C. verum*, *E. globulus*, *I. verum*, and *Z. limonella* were analyzed by gas chromatography-mass spectrometry (an Agilent Technology (USA) GC-MS system)^{10,27} at the Scientific Instrument Center of KMITL. Every chemical constituent was identified with Agilent software (version G1701DA D.00.00). The identity of each identified chemical by GC-MS was then confirmed by a retention index comparison. The retention index (RI) of each ingredient was calculated with respect to the homologous series of *n*-alkanes (C₇-C₃₀). That obtained RI of each EO constituent was compared to the reference RI for that constituent, reported in the chemical literature⁶¹⁻⁶².

P. americana rearing

Twenty pairs of *P. americana* adults were obtained from the National Institute of Health, Department of Medical Sciences, Thailand's Ministry of Public Health (13.85298° N latitude, 100.53008° E longitude). They were reared in an insectary laboratory, Faculty of Agricultural Technology, KMITL. The environmental conditions of the laboratory were a temperature of 27-28°C and an RH of 68-70%. The *P. americana* adults were reared in black insectary boxes (ten pairs per one 20.0 cm × 30.0 cm × 12.0 cm box) and fed with 20 g of dog biscuit mixed with 10 g of powdered milk, and 10% glucose solution, supplemented with 5% multivitamin syrup solution soaked in sterile cotton sheets. After 180-240 days of rearing, they grew up through stages of their life cycle, from eggs (ootheca) to nymphs, then to adults. Five-day-old ootheca and two-month-old adults were used in ovicidal and repellent bioassays, respectively.

Ovicidal bioassay

The ovicidal activity provided by each EO formulation against *P. americana* eggs was determined by a topical application assay^{42,47}. Two ootheca were placed in a 5-cm petri dish. Two dishes were placed in a black insectary box (16.0 cm wide × 26.0 cm long × 7.0 cm high): a treatment dish and a non-treatment dish. The treatment dish held eggs topically treated with 100 µL of each EO formulation or 10% (w/v) cypermethrin in soybean oil and in ethyl alcohol solvents, while the non-treatment dish held eggs topically pipetted with 100 µL of clean water. Each treatment was replicated five times. The number of hatched eggs was observed and recorded after 30 days of incubation (under the conditions of 27-28°C and 68-70% RH). The egg inhibition rate was calculated by the following formula⁴¹,

$$\text{Inhibition rate (\%)} = \left[\frac{U - T}{U} \right] \times 100, \quad (1)$$

where U is the total number of hatched eggs that were not treated with an active substance; T is the total number of hatched eggs treated with an EO solution or cypermethrin.

An inhibition rate index, as defined by Aungtikun and Soonwera²⁷ as an index of efficacy comparison between an EO and cypermethrin, was calculated by the formula below,

$$\text{Inhibition Rate Index (IRI)} = \frac{\% \text{ Inhibition rate of each EO formulation}}{\% \text{ Inhibition rate of cypermethrin}} \quad (2)$$

IRI < 1 signifies that the EO formulation was less toxic than cypermethrin; IRI = 1 signifies that the EO formulation was equally toxic to cypermethrin; and IRI > 1 signifies that the EO formulation was more toxic than cypermethrin.

Repellent activity bioassay

The repellent activity of each EO formulation against *P. americana* adults was determined by a dual-choice application assay⁴⁷. In the assay, each treatment formulation was dropped onto, absorbed by, and contained in half pieces of Whatman No.1® filter paper (18.0 cm wide × 28.0 cm long). One piece was a non-treatment area for comparing with another piece of treatment area. Two mL of clean water were dropped onto a non-treatment piece, and two mL of each treatment formulation were dropped onto a treatment piece. Both

pieces were placed in a black insectary box (20.0 cm wide × 30.0 cm long × 12.0 cm high) in separate areas: treatment and non-treatment areas. Two plastic cups (5.0 cm in diameter × 4.0 cm high) containing food (2 g of dog biscuits) and drink (50 mL of 10% glucose solution) for the cockroaches were placed on top of each treatment and non-treatment filter paper piece. Five male adults and five female adults of *P. americana* were released at the center of the insectary box. Either in soybean oil or ethyl alcohol solvent, 10% w/v cypermethrin was used as a positive control for the corresponding treatments of every EO in soybean oil and in ethyl alcohol.

Each experiment was repeated ten times. The number of cockroaches situated on the non-treatment area was observed and recorded at 1, 6, 12, 24, and 48 h. The repellent rate was calculated by the following formula¹⁸,

$$\text{Repellent rate} = \left[\frac{N-T}{N+T} \right] \times 100, \quad (3)$$

where T is the number of *P. americana* situated on the treatment area at the time of observation, and N is the number of *P. americana* situated on the non-treatment area.

Effective repellent index was calculated by the following formula¹⁸,

$$\text{Effective repellent index} = \frac{\% \text{ repellent of each EO formulation}}{\% \text{ repellent of cypermethrin}} \quad (4)$$

ERI <1 signifies that the EO formulation was less repellent against *P. americana* than cypermethrin; ERI = 1 signifies that the EO formulation was equally repellent to cypermethrin; and ERI >1 signifies that the EO formulation was more repellent than cypermethrin.

Statistical analysis

The numbers of hatched eggs and unhatched eggs in the treatment and non-treatment areas were analyzed for significant differences at $P < 0.05$ by a paired *t*-test method. The repellent activity assay was a completely randomized design. Significance differences at $P < 0.01$ were determined by ANOVA (one-way analysis of variance) and Duncan's Multiple Range Test (DMRT). The coefficients of the regression equation of time versus % repellent activity were determined by SPSS statistical software.

Declarations

Acknowledgements

This work was supported by King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520, Thailand, in a grant for KMITL Doctoral Scholarships (Grant No. KDS 2021/002 and 2560-01-04-002). We thank The Department of Medical Sciences, National Institute of Health, Thailand's Ministry of Public Health, for providing the adults of *P. americana* to us. Finally, we are grateful to Mr. Pratana Kangsadal, a KMITL Proofreader, for reviewing the English of the manuscript.

Author Contributions

This study was a collaboration of four researchers, each of whose contribution is described below:

- Mayura Soonwera: conceptualizing and supervising the study, writing review, and editing.
- Tanapoom Mounghipmalai: setting up the experiment; writing and editing the original draft.
- Wacharaporn Takawirapat: conducting the experiment and statistically analyzing the outcomes.
- Sirawut Sittichok: managing the experiment in general; writing and editing the original draft.

Competing Interests

The authors declare no competing interests.

References

1. World Health Organization (WHO). Cockroaches. www.who.int (2021).

2. Rogers, K. Encyclopaedia britannica: cockroach (Order Blattodea). IOP Publishing Physics Web. <https://www.britannica.com/animal/cockroach-insect> (2021).
3. Barbara, K.A. *et al.* American cockroach update 2017. IOP Publishing Physics Web. <https://www.entnemdept.ufl.edu/creatures/urban/roaches/American-cockroach.htm> (2017).
4. Borah, N. & Hazarika, L.K. Biology and morphometrics of *Periplaneta americana*. *J. Entomol. Zool. Stud.* **7**, 1206-1210 (2019).
5. Donkor, E.S. Nosocomial pathogens: an in-depth analysis of the vectorial potential of cockroaches. *Trop. Med. Infect. Dis.* **4**, 14. <https://doi.org/10.3390/tropicalmed4010014> (2019).
6. Donkor, E.S. Cockroaches and food-borne pathogens. *Environ. Health Insights.* **14**, 1-6. <https://doi.org/10.1177/1178630220913365> (2020).
7. Naher, A., Afroz, S. & Hamid, S. Cockroach associated foodborne pathogens: distribution and antibiogram. *Bangladesh Med. Res. Counc. Bull.* **44**, 30-38. <http://dx.doi.org/10.3329/bmrcb.v44i1.36802> (2018).
8. Moges, F. *et al.* Cockroaches as a source of high bacterial pathogens with multidrug resistant strains in Gondar Town, Ethiopia. *Biomed. Res. Int.* ID: 2825056. <http://dx.doi.org/10.1155/2016/2825056> (2016).
9. Rahimian, A.A. *et al.* A review on the insecticide resistance of three species of cockroaches (Blattodea: Blattidae) in Iran. *J. Econ. Entomol.* **112**, 1-10. <https://doi.org/10.1093/jee/toy247> (2018).
10. Zahraei-Ramazani, A.R., Saghaipour, A. & Vatandoost, H. Control of american cockroach (*Periplaneta americana*) in municipal sewage disposal system, Central Iran. *J. Arthropod-Borne Dis.* **12**, 172-179. <https://doi.org/10.18502/jad.v12i2.43> (2018).
11. Dingha, B.N. *et al.* Integrated pest management of the german cockroach (Blattodea: Blattellidae) in manufactured homes in rural North Carolina. *Fla. Entomol.* **99**, 587-592. <https://doi.org/10.1653/024.099.0401> (2016).
12. Hassaan, M.A. & El Nemr, A. Pesticides pollution: classifications, human health impact, extraction and treatment techniques. *Egypt J. Aquat. Res.* **46**, 207-220. <https://doi.org/10.1016/j.ejar.2020.08.007> (2020).
13. Eddleston, M. Poisoning by pesticides. *Medicine* **48**, 214- 217. <https://doi.org/10.1016/j.mpmed.2019.12.019> (2020).

14. Hikal, W.M., Baeshen, R.S. & Said-Al Ahl, H.A.H. Botanical insecticide as simple extractives for pest control. *Cogent Biol.* **3**, 1404274. <https://doi.org/10.1080/23312025.2017.1404274> (2017).
15. Gahukar, R.T. Use of plant-derived products to control household and structural arthropod pests. *Int. J. Basic Appl. Sci.* **6**, 22-28. <https://doi.org/10.14419/ijbas.v6i2.7483> (2017).
16. Zibae, I. & Khorram, P. Synergistic effect of some essential oils on toxicity and knockdown effects, against mosquitos, cockroaches and housefly. *Arthropods* **4**, 107-123. ISSN2224-4255 (2015).
17. Ebadollahi, A., Ziaee, M. & Palla, F. Essential oils extracted from different species of the lamiaceae plant family as prospective bioagents against several detrimental pests. *Molecules* **25**, 1556. <https://doi.org/10.3390/molecules25071556> (2020).
18. Ubulom, P.M.E., Yaro, C.A. & Udoh, U.A.P. Repellency and insecticidal properties of seed oil of *Jatropha curcas* L. against american cockroach, *Periplaneta americana* L. *J. Basic Appl. Zool.* **82**, 8. <https://doi.org/10.1186/s41936-021-00208-z> (2021).
19. Nour, A.H., Yap, S.S. & Nour, A.H. Extraction and chemical compositions of ginger (*Zingiber officinale* Roscoe) essential oils as cockroaches repellent. *Aust. J. Basic Appl. Sci.* **11**, 1-8 (2017).
20. Sittichok, S., Phaysa, W. & Soonwera, M. Repellency activity of essential oils on Thai local plants against american cockroach (*Periplaneta americana* L.; Blattidae: Blattodea). *Int. J. Agric. Technol.* **9**, 1613-1620 (2013).
21. Manzoor, F. *et al.* Efficacy of some essential oils against american cockroach *Periplaneta americana* (L.). *J. Med. Plant Res.* **6**, 1065-1069. <https://doi.org/10.5897/JMPR11.1244> (2012).
22. Gomes da Rocha Voris, D. *et al.* Evaluation of larvicidal, adulticidal, and anticholinesterase activities of essential oils of *Illicium verum* Hook. f., *Pimenta dioica* (L.) Merr., and *Myristica fragrans* Houtt. against zika virus vectors. *Environ. Sci. Pollut. Res.* **25**, 22541-22551. <https://doi.org/10.1007/s11356-018-2362-y> (2018).
23. Bellassoued, K. Protective effect of essential oil of *Cinnamomum verum* bark on hepatic and renal toxicity induced by carbon tetrachloride in rats. *Appl. Physiol. Nutr. Metab.* **44**, 606-618. <https://doi.org/10.1139/apnm-2018-0246> (2019).
24. Soonwera, M. & Sittichok, S. Adulticidal activities of *Cymbopogon citratus* (Stapf.) and *Eucalyptus globulus* (Labill.) essential oils and of their synergistic combinations against *Aedes aegypti* (L.), *Aedes albopictus* (Skuse), and *Musca domestica* (L.). *Environ. Sci. Pollut. Res.* **27**, 20201-20214. <https://doi.org/10.1007/s11356-020-08529-2> (2020).
25. Joshi, A. *et al.* A comparative study of the chemical composition of the essential oil from

Eucalyptus globulus growing in Dehradun (India) and around the world. *Orient J. Chem.* **32**, 331-340. <http://dx.doi.org/10.13005/ojc/320137> (2016).

26. Charoensup, R. *et al.* Pharmacognostic specification of *Zanthoxylum limonella* (Dennst.)

Alston: fruits and seeds in Thailand. *J. Adv. Pharm. Technol. Res.* **7**, 134-138. <https://doi.org/10.4103/2231-4040.191421> (2016).

27. Aungtikun, J. & Soonwera, M. Improved adulticidal activity against *Aedes aegypti* (L.) and

Aedes albopictus (Skuse) from synergy between *Cinnamomum* spp. essential oils. *Sci. Rep.*

11, 4685. <https://doi.org/10.1038/s41598-021-84159-z> (2021).

28. Aungtikun, J., Soonwera, M. & Sittichok, S. Insecticidal synergy of essential oils from

Cymbopogon citratus (Stapf.), *Myristica fragrans* (Houtt.), and *Illicium verum* Hook. f. and their major active constituents. *Ind. Crops Prod.* **164**, 113386.

<https://doi.org/10.1016/j.indcrop.2021.113386> (2021).

29. Soonwera, M. & Phasomkusolsil, S. Adulticidal, larvicidal, pupicidal and oviposition deterrent

activities of essential oil from *Zanthoxylum limonella* Alston (Rutaceae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Asian Pac. J. Trop. Biomed.* **7**, 967-978. <https://doi.org/10.1016/j.apjtb.2017.09.019> (2017).

30. Verma, R.K. *et al.* Evaluation of essential oil yield and chemical composition of eight

lemongrass (*Cymbopogon* spp.) cultivars under Himalayan region. *J. Essent. Oil Res.*

27, 197-203. <https://doi.org/10.1080/10412905.2015.1014936> (2015).

31. Li, Y.Q., Kong, D.X. & Wu, H. Analysis and evaluation of essential oil components of

cinnamon barks using GC-MS and FTIR spectroscopy. *Ind. Crops Prod.* **41**, 269-278.

<https://doi.org/10.1016/j.indcrop.2012.04.056> (2013).

32. Sebei, K. *et al.* Chemical composition and antibacterial activities of seven *Eucalyptus* species

essential oils leaves. *Bio. Res.* **48**, 7. <https://doi.org/10.1186/0717-6287-48-7> (2015).

33. Gholivand, M.B., Rahimi-Nasrabadi, M. & Chalabi, H. Determination of essential oil

components of star anise (*Illicium verum*) using simultaneous hydrodistillation–static

headspace liquid-phase microextraction–gas chromatography mass spectrometry. *Anal. Lett.* **42**, 1382-1397.

<https://doi.org/10.1080/00032710902961081> (2009).

34. Chauhan, N., Malik, A. & Sharma, S. Repellency potential of essential oils against housefly,

Musca domestica L. *Environ. Sci. Pollut. Res.* **25**, 4707-4714.

<https://doi.org/10.1007/s11356-017-0363-x> (2018).

35. Chansang, A. *et al.* Potential of natural essential oils and cinnamaldehyde as insecticides

against the dengue vector *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian J. Trop.*

Med. Public Health **49**, 6-22 (2018).

36. Cotchakaew, N. & Soonwera, M. Toxicity of several botanical essential oils and their combinations against females of *Aedes albopictus* (Skuse) and *Anopheles minimus* (Theobald): oviposition deterrent, ovicidal and adulticidal efficacies. *Asian Pac. J. Trop. Biomed.* **9**, 29-39. <https://doi.org/10.4103/2221-1691.250267> (2019).
37. Junior, G.S.L. *et al.* Chemical composition and acaricidal activity of essential oils from fruits of *Illicium verum* and rhizomes of *Curcuma zedoaria* against *Dermacentor nitens* (Acari: Ixodidae). *J. Essent. Oil Res.* **32**, 571-576. <https://doi.org/10.1080/10412905.2020.1804002> (2020).
38. Imphat, C. & Woottisin, N. The original articles development of massage oil containing essential oil from *Zanthoxylum limonella* fruit and pain relief effect on calf muscles in healthy volunteers. *J. Thai Trad. Alt. Med.* **19**, 148-160 (2021).
39. Wongkattiya, N. *et al.* Chemical compositions and biological properties of essential oils from *Zanthoxylum rhetsa* (Roxb.) DC and *Zanthoxylum limonella* Alston. *Afr. J. Tradit. Complement Altern. Med.* **15**, 12-18. <https://doi.org/10.21010/ajtcamv15i2.2> (2018).
40. Sriwichai, T. *et al.* *Zanthoxylum* spp.: a new potential sources of essential oil for the perfumery and pharmaceutical industries in Thailand. *Med. Plants - Int. J. Phytomed. Relat. Ind.* **11**, 26-45. <https://doi.org/10.5958/0975-6892.2019.00009.1> (2019).
41. Soonwera, M., Wongnet, O. & Sittichok, S. Ovicidal effect of essential oils from Zingiberaceae plants and *Eucalytus globulus* on eggs of head lice, *Pediculus humanus capitis* De Geer. *Phytomedecine* **47**, 93-104. <https://doi.org/10.1016/j.phymed.2018.04.050> (2018).
42. Sinthusiri, J. & Soonwera, M. Oviposition deterrent and ovicidal activities of seven herbal essential oils against female adults of housefly, *Musca domestica* L. *Parasitol. Res.* **113**, 3015-3022. <https://doi.org/10.1007/s00436-014-3964-z> (2014).
43. Matos, L.S. *et al.* Chemical composition and insecticidal effect of essential oils from *Illicium verum* and *Eugenia caryophyllus* on *Callosobruchus maculatus* in cowpea. *Ind. Crop Prod.* **145**, 12088. <https://doi.org/10.1016/j.indcrop.2020.112088> (2020).
44. Marsin, A.M. *et al.* Essential oils as insect repellent agents in food packaging: a review. *Eur. Food Res. Technol.* **246**, 1519-1532. <https://doi.org/10.1007/s00217-020-03511-1> (2020).
45. Naveenkumar, P.B. *et al.* Toxicity and repellency of essential oils against rice weevil *Sitophilus oryzae* (L.). *Indian J. Entomol.* **81**, 575-584. <https://doi.org/10.5958/0974-8172.2019.00122.6> (2019).
46. Kasim, N.N. *et al.* Extraction and potential of cinnamon essential oil towards repellency and insecticidal activity. *Int. J. Sci. Res.* **4**, 1-6 (2014).
47. Sittichok, S. & Soonwera, M. Repellency activity and oothecal toxicity of eight herbal essential

oils against american cockroach, *Periplaneta americana* L. (Blattidae: Blattodea).

Proceeding of the 51st Kasetsart university annual conference, Bangkok, Thailand, pp 206-213 (2013).

48. Soonwera, M. & Phasomkusosil, S. Efficacy of Thai herbal essential oils as green repellent against mosquito vectors. *Acta. Trop.* **142**, 127-130.

<https://doi.org/10.1016/j.actatropica.2014.11.010> (2015).

49. Phasomkusosil, S. & Soonwera, M. Insect repellent activity of medicinal plant oils against *Aedes*

aegypti (Linn.), *Culex quinquefasciatus* Say and *Anopheles minimus* (Theobald) based on protection time and biting rate. *Southeast Asian J. Trop. Med. Public. Health* **41**, 831-840 (2010).

50. Phukerd, U. & Soowera, M. Repellency of essential oils extracted from Thai native plants

against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Parasitol. Res.* **113**, 3333-

3340. <https://doi.org/10.1007/s00436-014-3996-4> (2014).

51. Campbell, B.E. & Miller, D.M. Insecticide resistance in eggs and first instars of the bed bug,

Cimex lectularius (Hemiptera: Cimicidae). *Insects* **6**, 122-132.

<https://doi.org/10.3390/insects6010122> (2015).

52. Campbell, B.E., Pereira, R.M. & Koehler, P.G. Complications with controlling insect eggs.

IOP Publishing Physics Web. <https://www.intechopen.com/chapters/49557> (2016).

53. Rajashekar, Y. & Shivanandappa, T. Mode of action of the natural insecticide, decaleside

involves sodium pump inhibition. *PLoS One* **12**, e0170836.

<https://doi.org/10.1371/journal.pone.0170836> (2017).

54. Jankowska, M. *et al.* Molecular targets for components of essential oils in the insect nervous

system-A review. *Molecules* **23**, 34. <https://doi.org/10.3390/molecules23010034> (2018).

55. Pavela, R. Insecticidal properties of *Pimpinella anisum* essential oils against the *Culex*

quinquefasciatus and the non-target organism *Daphnia magna*. *J. Asia-Pac. Entomol.*

17, 287-293. <http://dx.doi.org/10.1016/j.aspen.2014.02.001> (2014).

56. Bosch-Serra, D. *et al.* Esterase, glutathione s-transferase and NADPH-cytochrome P450

reductase activity evaluation in *Cacopsylla pyri* L. (Hemiptera: Psyllidae) individual adults. *Insects* **12**, 329.

<https://doi.org/10.3390/insects12040329> (2021).

57. Devi, K.C. & Devi, S.S. Insecticidal and oviposition deterrent properties of some spices against

coleopteran beetle, *Sitophilus oryzae*. *J. Food Sci. Technol.* **50**, 600-604.

<https://doi.org/10.1007/s13197-011-0377-1> (2013).

58. Ichikawa, H. Neurotoxicology of pesticides. *Brain Nerve*. **67**, 39-48.

<https://doi.org/10.11477/mf.1416200085> (2015).

59. Sharma, A. *et al.* Cypermethrin toxicity: a review. *J. Forensic Sci. & Criminal Inves.*

9, 555767. <https://doi.org/10.19080/JFSCI.2018.09.555767> (2018).

60. Patra, J.K. *et al.* Star anise (*Illicium verum*): chemical compounds, antiviral properties, and

clinical relevance. *Phytother. Res.* **34**, 1248-1267. <https://doi.org/10.1002/ptr6614> (2020).

61. Adams, R. Identification of essential oil components by gas chromatography/mass

spectrometry, 4th edition. Allured Publishing Corp. Carol Stream (2007), ISBN-13:979-

1932633214.

62. NIST 17. The NIST 17 Mass spectral library (NIST2017/EPA/NIH). National Standards

and Technology, Gaithersburg (2017).

Tables

Item	Compound	R ^a	K ^b	Peak area (%)					I ^c
				<i>C. citratus</i>	<i>C. verum</i>	<i>E. globulus</i>	<i>I. verum</i>	<i>Z. limonella</i>	
1.	Acetoin	680	680	-	-	-	0.41	-	M,R
2.	α-Thujene	928	930	-	-	-	0.21	2.31	M,R
3.	α-Pinene	932	933	3.52	0.91	5.01	0.32	1.83	M,R
4.	Camphene	946	946	-	0.52	0.32	-	-	M,R
5.	Sabinene	977	977	-	-	3.75	-	4.52	M,R
6.	B-Mycene	990	991	-	0.43	0.31	-	0.32	M,R
7.	α-Phellandrene	1002	1003	-	0.32	1.67	-	1.31	M,R
8.	α-Terpinene	1002	1002	0.22	-	-	0.31	4.82	M,R
9.	Benzene	1011	1009	-	14.15	8.32	-	8.21	M,R
10.	1,8-Cineole	1024	1025	10.93	0.61	45.82	0.52	-	M,R
11.	Limonene	1029	1029	-	-	-	1.85	28.13	M,R
12.	γ-Terpinene	1051	1050	0.26	-	20.13	-	7.82	M,R
13.	Butanoic acid	1085	1086	-	-	0.52	-	-	M,R
14.	Linalool	1086	1086	0.86	-	-	-	1.15	M,R
15.	Terpinolene	1089	1088	-	-	1.12	0.23	2.23	M,R
16.	D-Fenchyl alcohol	1110	1110	-	-	0.55	-	-	M,R
17.	<i>trans</i> -Pinocaveol	1139	1139	-	-	0.72	-	-	M,R
18.	Borneol	1146	1147	-	1.23	0.81	-	-	M,R
19.	Terpinen-4-ol	1165	1166	-	-	3.21	-	24.13	M,R
20.	<i>trans</i> -Carveol	1204	1204	-	-	0.23	-	1.73	M,R
21.	Myrtenol	1214	1214	-	-	0.71	-	-	M,R
22.	Neral	1217	1217	25.72	-	-	-	-	M,R
23.	Cinnamaldehyde	1222	1222	-	75.23	-	-	-	M,R
24.	<i>p</i> -Anisaldehyde	1223	1223	-	-	-	1.63	-	M,R
25.	Carvone	1230	1231	-	-	-	-	2.47	M,R
26.	Geraniol	1237	1237	5.71	-	-	-	-	M,R
27.	Geranial	1247	1247	46.33	-	-	-	-	M,R
28.	α-Terpineol	1280	1279	-	-	2.85	-	6.23	M,R
29.	<i>trans</i> -Anethole	1283	1285	-	-	-	92.24	-	M,R
30.	Eugenol	1356	1355	-	-	-	0.84	-	M,R
31.	Geranyl acetate	1379	1380	3.87	-	-	-	-	M,R
32.	Copaene	1380	1381	-	2.02	-	-	-	M,R
33.	Cinnamic acid	1460	1462	-	0.33	-	-	-	M,R
34.	α-Gurjunene	1527	1529	-	-	0.86	-	-	M,R
35.	Epiglobulol	1563	1564	-	-	0.44	-	-	M,R

36.	Caryophyllene	1578	1580	1.17	-	-	-	0.54	M,R
37.	Cadalene	1659	1659	-	0.32	-	-	-	M,R
Total (%)				97.40	98.60	97.35	98.56	97.75	-
Yield (%)				1.15	1.10	1.21	3.23	9.27	-

Table 1 Chemical constituents of *Cymbopogon citratus*, *Cinnamomum verum*, *Eucalyptus globulus*, *Illicium verum*, and *Zanthoxylum limonella* and their percentage yield in the total chemical profile. ^aR = Retention index of a chemical constituent determined with an HP-5 MS column and compared with the retention indices of standard alkanes (C₇-C₃₀) for identity verification. ^bK = Kovat retention index from NIST17⁶². ^cI = Identified and confirmed by mass spectrum (M) matching with chemicals in the computer mass library of Adams⁶¹ and by retention index (R) matching with those reported in NIST17⁶².

Treatment	Solvent	Number of hatched eggs ±SD		Inhibition rate (%)	P-value	IRI
		Treated	Untreated			
<i>C. citratus</i> EO	S	12.8±1.9*	145.1±5.3	91.2	0.03	0.91
	E	55.7±4.8	140.3±6.4	60.3	0.08	0.60
<i>C. verum</i> EO	S	20.3±3.4*	138.2±6.7	85.3	0.04	0.85
	E	27.5±4.8*	140.5±7.4	80.4	0.10	0.80
<i>E. globulus</i> EO	S	16.2±2.7*	128.7±5.9	87.4	0.03	0.87
	E	40.7±4.8	130.8±5.3	68.9	0.10	0.69
<i>I. verum</i> EO	S	3.9±1.8*	125.7±2.8	96.9	0.01	0.97
	E	18.8±2.7*	130.7±3.9	85.6	0.06	0.86
<i>Z. limonella</i> EO	S	9.6±1.9*	132.4±5.5	92.7	0.01	0.93
	E	23.7±4.3*	137.2±4.8	82.7	0.05	0.83
cypermethrin	S	0*	148.4±3.7	100	0.01	-
	E	0*	152.3±6.8	100	0.01	-

Table 2 Inhibition rate at 30 days against *Periplaneta americana* eggs of 10% solutions of five plant essential oils (EOs) and cypermethrin in soybean oil and in ethyl alcohol. *IRI* Inhibition Rate Index, *S* Soybean oil solution, *E* Ethyl alcohol solution, *Treatment and non-treatment are significantly different at *P*<0.05.

Treatment	Solvent	% Repellent / Time (h)					Regression equation of time versus % repellent	R^2
		1	6	12	24	48		
<i>C. citratus</i> EO	S	98.0±4.0 ^a	93.8±5.1 ^a	92.8±6.1 ^a	92.0±6.8 ^a	79.0±1.9 ^a	y= -0.1228x+93.257	0.94
	E	88.2±5.9 ^{ab}	78.4±3.6 ^b	78.0±2.4 ^b	69.6±1.8 ^b	62.0±2.4 ^b	y= -0.1735x+92.192	0.92
<i>C. verum</i> EO	S	100 ^a	91.0±3.7 ^a	88.6±1.9 ^a	88.4±1.7 ^a	83.4±3.4 ^a	y= -0.0967x+93.764	0.94
	E	84.8±4.5 ^b	80.2±2.4 ^{ab}	75.6±4.6 ^b	73.0±3.3 ^b	62.8±4.3 ^b	Y=-0.1134x+94.768	0.92
<i>E. globulus</i> EO	S	80.2±1.3 ^{bc}	72.1±3.1 ^b	68.2±1.9 ^{bc}	64.4±4.1 ^{bc}	61.6±2.7 ^b	y= -0.1614x+89.732	0.89
	E	71.6±3.4 ^c	63.9±2.3 ^c	62.8±2.4 ^c	59.2±1.1 ^c	57.2±2.3 ^{bc}	Y=-0.1829x+86.831	0.87
<i>I. verum</i> EO	S	82.6±4.4 ^{bc}	79.0±4.2 ^b	76.4±3.7 ^b	74.2±2.1 ^b	66.0±3.7 ^b	y= -0.0973x+84.119	0.81
	E	74.4±4.6 ^c	69.6±3.3 ^{bc}	65.8±2.1 ^c	62.0±2.4 ^{bc}	55.3±4.5 ^{bc}	y= -0.1193x+83.802	0.84
<i>Z. limonella</i> EO	S	87.2±3.7 ^{bc}	80.6±2.6 ^{ab}	77.6±1.8 ^b	72.4±2.2 ^b	60.8±5.1 ^b	Y=-0.1795x+84.648	0.85
	E	83.8±3.8 ^b	75.6±3.4 ^b	69.6±1.5 ^{bc}	59.8±4.1 ^c	47.4±6.1 ^c	Y=0.1853x+80.167	0.81
cypermethrin	S	73.4±3.1 ^c	64.2±4.1 ^c	63.0±2.4 ^c	56.6±2.9 ^c	48.8±5.9 ^c	y= -0.2017x+88.431	0.89
	E	69.6±3.3 ^c	63.5±2.2 ^c	62.4±2.2 ^c	56.0±2.8 ^c	43.6±5.2 ^c	y= -0.2306x+90.715	0.92
<i>F-test, Df_{total}</i>		^{**} ,119	^{**} ,119	^{**} ,119	^{**} ,119	^{**} ,119		

Table 3 Repellent activities against *Periplaneta americana* adults of 10% solutions of five plant essential oils (EOs) and cypermethrin in soybean oil and in ethyl alcohol. Mean percentage repellent activity within the same column followed by different letters are significantly different at $P<0.01$ (ANOVA and Duncan's Multiple Range Test). *S* Soybean oil formulation, *E* Ethyl alcohol formulation, R^2 Regression coefficient, ^{**}Significantly different at $P<0.01$.

Treatment	Solvent	Effective repellent index (ERI) / Time (h)				
		1	6	12	24	48
<i>C. citratus</i> EO	S	1.33	1.46	1.47	1.63	1.62
	E	1.27	1.23	1.42	1.24	1.42
<i>C. verum</i> EO	S	1.36	1.42	1.41	1.56	1.71
	E	1.23	1.25	1.21	1.30	1.44
<i>E. globulus</i> EO	S	1.09	1.13	1.08	1.14	1.26
	E	1.03	1.01	1.01	1.14	1.31
<i>I. verum</i> EO	S	1.17	1.23	1.21	1.31	1.35
	E	1.07	1.09	1.05	1.11	1.23
<i>Z. limonella</i> EO	S	1.19	1.26	1.23	1.28	1.25
	E	1.20	1.18	1.12	1.07	1.09
cypermethrin	S	-	-	-	-	-
	E	-	-	-	-	-

Table 4 Effective repellent indices (ERI) against *Periplaneta americana* adults of 10% solutions of five plant essential oils (EOs) and in soybean oil and in ethyl alcohol. *S* Soybean oil formulation, *E* Ethyl alcohol formulation.

Treatment	Solvent	Number of hatched eggs $\pm$ SD		Inhibition rate (%)	P-value	IRI
		Treated	Untreated			
<i>I. verum</i> EO + <i>C. verum</i> EO	S	0.8 $\pm$ 0.2*	122.7 $\pm$ 2.1	99.3	0.01	0.99
	E	10.3 $\pm$ 1.9*	127.8 $\pm$ 5.1	91.9	0.05	0.92
cypermethrin	S	0*	148.4 $\pm$ 3.7	100	0.01	-
	E	0*	152.3 $\pm$ 6.8	100	0.01	-

Table 5 Inhibition rate against *Periplaneta americana* eggs at 30 days after treated with a combined formulation of 5% *I. verum* EO + 5% *C. verum* EO and 10% cypermethrin, both in soybean oil and in ethyl alcohol solvents. *IRI* Inhibition Rate Index, *S* Soybean oil solution, *E* Ethyl alcohol solution, *Treatment and non-treatment are significantly different at $P < 0.05$.

Treatment	Solvent	% Repellent / Time (h)					Regression equation of time versus % repellent	R^2	ERI at 48 h
		1	6	12	24	48			
<i>I. verum</i> EO + <i>C. verum</i> EO	S	100 ^a	95.7 $\pm$ 3.1 ^a	94.2 $\pm$ 4.2 ^a	93.3 $\pm$ 3.4 ^a	89.5 $\pm$ 3.1 ^a	Y=-0.0898x+94.725	0.95	1.94
	E	90.3 $\pm$ 3.7 ^a	87.2 $\pm$ 4.3 ^b	80.7 $\pm$ 3.6 ^b	78.2 $\pm$ 4.3 ^b	65.7 $\pm$ 2.8 ^b	Y=0.1853x+80.167	0.81	1.47
cypermethrin	S	70.3 $\pm$ 3.4 ^c	62.4 $\pm$ 3.7 ^c	60.5 $\pm$ 2.7 ^c	58.4 $\pm$ 2.1 ^c	46.2 $\pm$ 3.3 ^c	y=-0.2115x+89.337	0.87	-
	E	69.7 $\pm$ 3.1 ^c	60.3 $\pm$ 3.1 ^c	58.2 $\pm$ 2.9 ^c	55.3 $\pm$ 3.8 ^c	44.7 $\pm$ 4.2 ^c	y=-0.2215x+88.863	0.89	-
<i>F</i> -test, <i>Df</i> _{total}		** ,39	** ,39	** ,39	** ,39	** ,39			

Table 6 Repellent activities against *Periplaneta americana* adults of a combined formulation of 5% *I. verum* EO + 5% *C. verum* EO and 10% cypermethrin, both in soybean oil and ethyl alcohol solvents. Mean percentage repellent activity within the same column followed by different letters are significantly different at $P < 0.01$ (ANOVA and Duncan's Multiple Range Test). *ERI* Effective repellent index, *S* Soybean oil formulation, *E* Ethyl alcohol formulation, R^2 Regression coefficient, **Significantly different at $P < 0.01$.

Figures

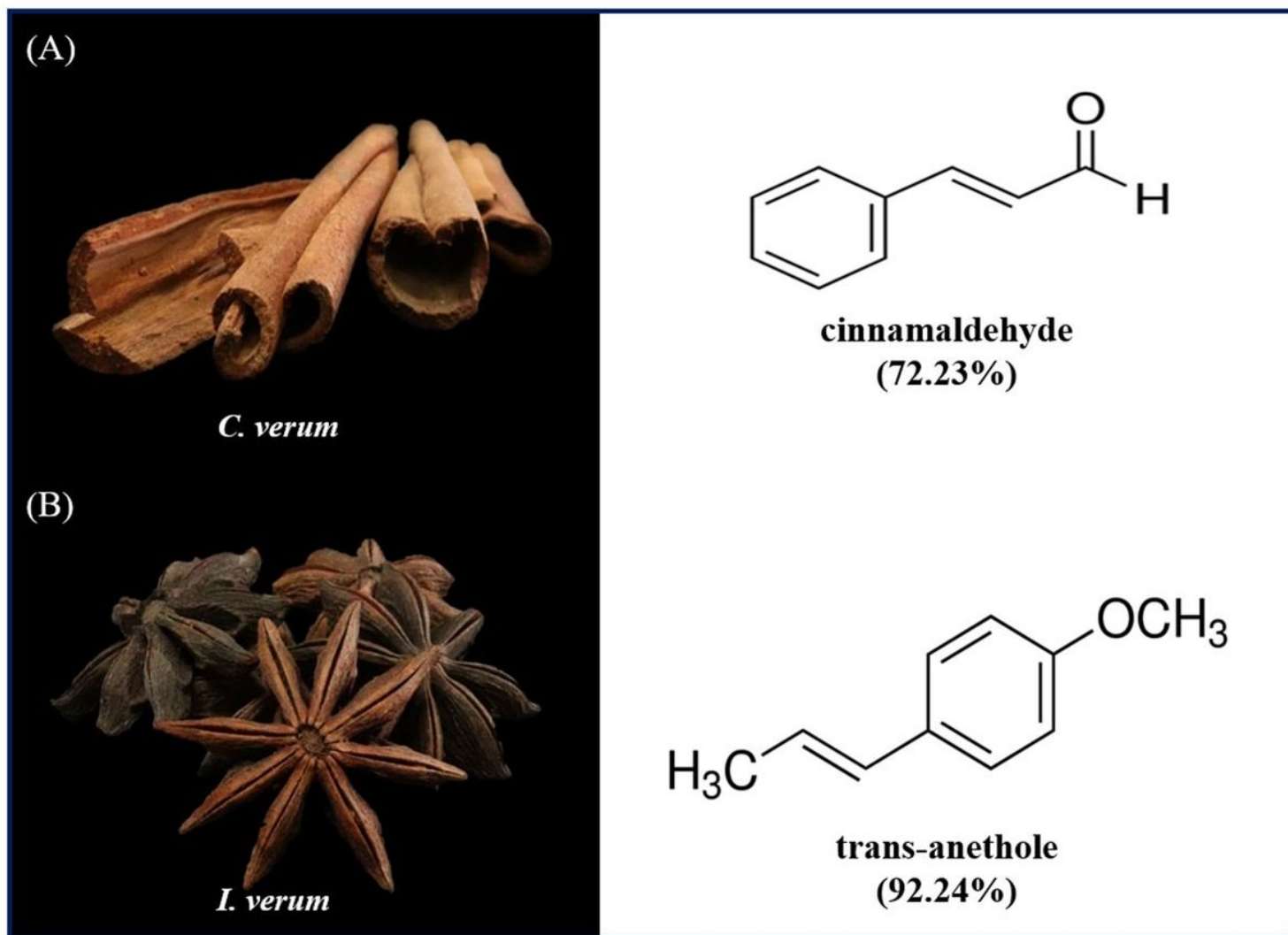


Figure 1

Photos of *Cinnamomum verum* bark (A) and *Illicium verum* fruit (B) and the chemical structures of their major essential oil (EO) constituent.

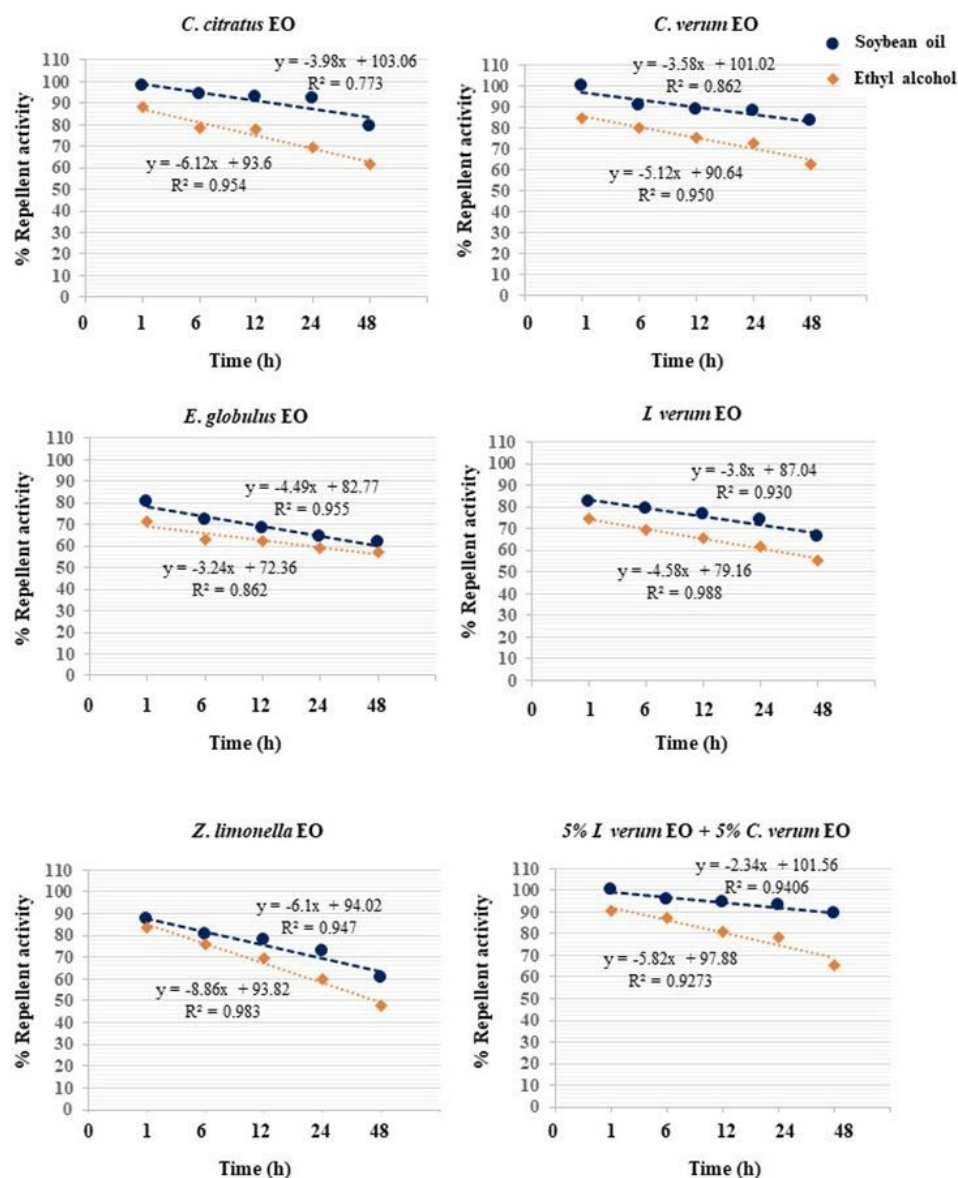


Figure 2

Relationships between percentage repellent activity and time (h) of five essential oils (EOs) and a combined formulation of two EOs in soybean oil and in ethyl alcohol against *Periplaneta americana*.

Note: blue line represents the relationship between percentage repellent activity and time of an EO in soybean oil, while the orange line represents the same kind of relationship in ethyl alcohol.