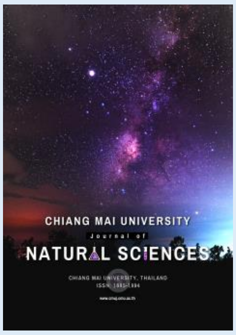

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Research article

The Repellent Potential of Herbal Oils Alone and in Combination in Mouse Behavioral Models (*Mus musculus*)

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Abstract Natural repellent products are of interest worldwide due to their low toxicity, rapid degradation, and being friendly to the environment. The current study evaluated the efficiency of herbal oils from fragrant plants as mouse repellents. Orange oil (*Citrus sinensis* (L.) Osbeck), peppermint oil (*Mentha × piperita* L.), lemongrass oil (*Cymbopogon citratus* (DC.) Stapf), ginger oil (*Zingiber officinale* Roscoe), plai oil (*Zingiber cassumunar* Roxb.), menthol and their combination were tested in a radial arm maze and light/dark transition test. For the short-term effect (week 0), each of the herbal oils and their combination were effective as mouse repellents, as shown by increased latency times and decreased time spent in arms containing repellents in the radial arm maze. Similarly, mice exposed to each of the herbal oils and their combination in the dark chamber spent more time in the light chamber in the light/dark transition test. Only peppermint oil and menthol failed to maintain their repellent activity in the long-term effect (week 1). Thus, we recommend spraying orange oil, lemongrass oil, ginger oil, plai oil and the combination of all oils every week for effective mouse repellant activity.

Keywords: Medicinal plant, Mice, Natural product, Essential oils, Anxiety

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INTRODUCTION

The common house mouse is considered an annoyance to humans since it eats and spoils stored food and contaminates living areas with its droppings and urine (Jokić et al., 2017). It is also a vector for a range of zoonotic agents that cause diseases that represent a threat to public health such as leptospirosis, plague, scrub typhus, murine typhus, hantavirus pulmonary syndrome, trichinosis and angiostrongyliasis (Meerburg et al., 2009: Jokić et al., 2017). Mice can also cause economic losses by damaging agricultural fields and disrupting transportation (Kalandakanond-Thongsong et al., 2010).

Today, the worldwide consumption of synthetic repellents is increasing as producers attempt to minimize losses in stored grains, fruits and other cellulosic materials by preventing pest and rodent infestations (Jokić et al., 2017). Most conventional pesticides are inherently toxic and damaging to the environment (Khater, 2012). Therefore, employing natural extracts with low toxicity as mouse repellents could be a better option. The repellant properties of essential oils from various plant species have been widely researched based on their strong odor (Singla et al., 2014). The aromatic oils of bergamot and lavender are commonly known active ingredients in commercial rodent and mosquito repellents (Jokić et al., 2017).

Several researchers have previously investigated the efficacy of natural compounds as insect and rodent repellents (Bari et al., 2020). Kalandakanond-Thongsong and colleagues (2010) investigated rat responses to wintergreen oil, chilli, peppermint oil, bergamot oil and geranium oil using a circular open field behavioral model. They discovered that each of the tested compounds had a significant repellent effect when compared to the control and this effect was maintained over a 7 day period (Kalandakanond-Thongsong et al., 2010). Singla and colleagues (2014) reported that eucalyptus oil acted as a repellent to house rats based on the reduced intake of food placed close to eucalyptus oil (Singla et al., 2014). Deletre and colleagues (2015) investigated the repellent effects of cinnamon (*Cinnamomum zeylanicum*), citronella (*Cymbopogon winternarius*), cumin (*Cuminum cyminum*) and lemongrass (*Cymbopogon citratus*) aromatic oils against *Bemisia tabaci* whiteflies. Two bioactive compounds derived from lemongrass, citronellol and geraniol, were discovered to have repellent properties (Deletre et al., 2016). Bari and colleagues (2020) used a choice test (T-maze arena) and a no-choice test (metabolic cage) to demonstrate the repellant efficacy of methanol extract of *Nerium oleander* leaves against rice-field rats. They discovered that rats avoided eating and drinking near the oleander extract treated area in the T-maze arena and showed reduced food consumption and feces deposition along with increased water consumption and urine production when exposed to oleander extract in the metabolic cage. Furthermore, *Nerium oleander* extract disrupted daily activity patterns; increasing resting activity and decreasing locomotion and nesting activity (Bari et al., 2020).

Extracts of many aromatic plants such as neem oil, lemongrass oil, black pepper oil, bergamot, fennel, peppermint oil, onion oil and plai oil can be used as insect and animal repellents according to Thai wisdom (Trongtokit et al., 2005). The purpose of this study was to evaluate the mouse repellent potential of orange oil (*Citrus sinensis* (L.) Osbeck), peppermint oil (*Mentha* × *piperita* L.), lemongrass oil (*Cymbopogon citratus* (DC.) Stapf), ginger oil (*Zingiber officinale* Roscoe), plai oil (*Zingiber cassumunar* Roxb.), and menthol using a radial arm maze and light/dark transition test.

MATERIAL AND METHODS

Materials

Commercial grade herbal oils and menthol were obtained from ARK@RICH (Samut Prakan, Thailand) without any quality control analysis. The combination was prepared by first combining 1 g of each herbal oil (orange oil, peppermint oil, lemongrass oil, ginger oil, and plai oil) then adding 1 g of menthol and thoroughly mixing with a vortex mixer until homogenized. Animal food was supplied by the Northeast Laboratory Animal Center (Khon Kaen, Thailand).

Animals

Seven-week-old male ICR mice (25-35 g) were provided by Nomura Siam International (Bangkok, Thailand). They were housed in the Laboratory Animal Unit of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand at 23 ± 2 °C with 45% ± 2% humidity. Light was controlled as twelve-hour light–dark cycles. The experimental procedures were approved as No. AEKKU 14/2560 by Khon Kaen University's Animal Ethics Committee for Use and Care.

Experimental design

Animals were randomly divided into eight groups (n=12 each) including one control group (water) and seven repellent test groups. The dose of herbal oils in this study was determined using an in-cage mosquito repellent bioassay (Ma et al., 2019). Short-term and long-term repellent effects were assessed using a radial arm maze and a light/dark transition test. The short-term repellent effect (week 0) was determined directly after spraying the herbal oils or their combination onto pieces of fabric that were then placed into the apparatus, whereas the long-term repellent effect was determined one week (week 1) after spraying the herbal oils or their combination onto the pieces of fabric (Kalandakanond-Thongsong et al., 2010). Mice were fasted before testing for 18 h to induce stress and hunger, as described in the food acceptance test (Li et al., 2019).

Behavioral models

Radial arm maze

The radial arm maze is an eight-armed array that radiates from a central starting point with a cup containing a food reward located at the end of each arm (Valladolid-Acebes et al., 2011). Alternate arms of the maze had pieces of fabric (size 5x10 cm) that had been sprayed with either 2 mL of a repellent sample or 2 mL of water placed at the entrance (Figure 1). A mouse was placed in the center of the apparatus and an investigator recorded the amount of time before the mouse entered each arm (latency time) and the total time spent in each arm over five minutes. In addition, locomotor activity was evaluated from the number of entries into each arm (Beninger et al., 2009).

Light/dark transition test

The light/dark transition test was performed as described previously by Khamphukdee et al. (2017) with some modifications. The apparatus consists of two chambers, one of which is larger and brighter, while the other is smaller and darker (Figure 2). Pieces of fabric (size 5x10 cm) that had been sprayed with either 2 mL of a repellent sample or 2 mL of water were placed in the dark chamber. Mice were placed in the dark chamber and the time spent in each chamber over a five minute test period was recorded.

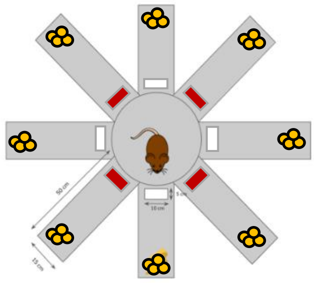

Figure 1. **Radial arm maze apparatus**

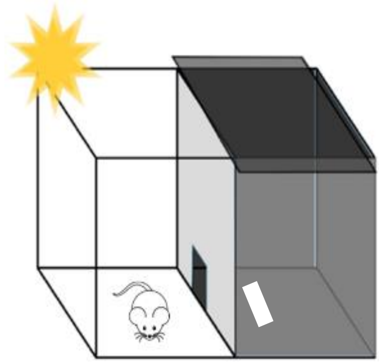

Figure 2. **Light/dark apparatus**

Statistical analysis

The collected data are reported as mean ± S.E.M and were analyzed using a one-way ANOVA followed by the Turkey test for multiple comparisons between groups. Statistically significant differences were defined as $P < 0.05$. SigmaStat® ver. 3.5 (Systat Software Inc., Richmond, CA, USA) was used to conduct the analysis.

RESULTS

Effect of herbal oils alone and in combination in the radial arm maze

The repellent activity of the herbal oils was evaluated based on the latency time and time spent in the arms of the radial arm maze. Increased latency time and decreased time spent in the arms indicate a mouse repellent effect (Witmer et al., 2014). All tested repellants showed significantly longer latency times than water in the short-term test (Figure 3A), with the combination spray showing the most repellant effect, followed by ginger oil, plai oil, orange oil, peppermint oil, lemongrass oil, and menthol. After one week (Figure 3B), the combination spray still showed the highest latency time and repellant effect, followed by ginger oil, plai oil, orange oil, and lemongrass oil. In contrast, peppermint oil and menthol showed no significant repellent effects after seven days.

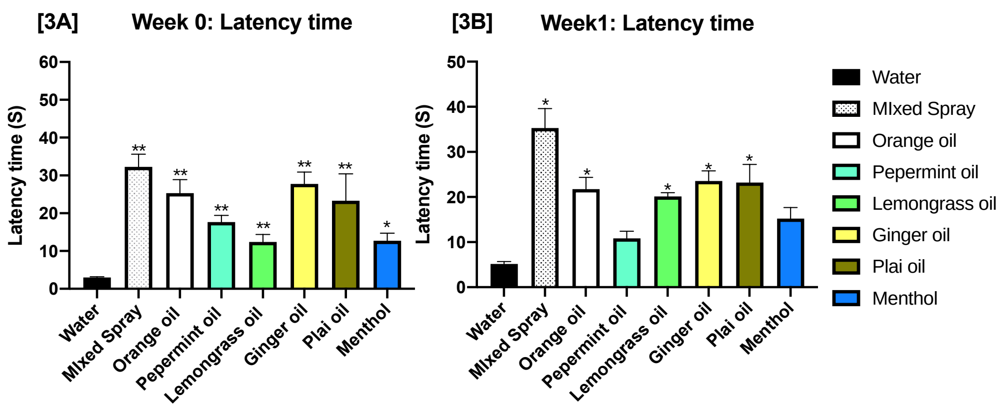

Figure 3. **The latency time to each arm.** The results are expressed as the mean ± SEM (n = 10-12). Week 0 is presented in Panel A, whereas week 1 is presented in Panel B. $**P < 0.001$ and $*P < 0.05$ vs water.

The average amount of time that mice spend in each arm in the radial arm maze reflects the repellant potential of the test substance. In the short-term test (Figure 4A), mice spent significantly less time in the arms containing each of the repellents compared to water. Mice spent the shortest time in arms containing the combination spray followed by lemongrass oil, menthol, plai oil, ginger oil, orange oil and peppermint oil. After one week (Figure 4B), the combination spray still showed the shortest time spent, followed by lemongrass oil, plai oil, ginger oil, and orange oil. In contrast, peppermint oil and menthol showed no significant repellant effects after seven days compared to water.

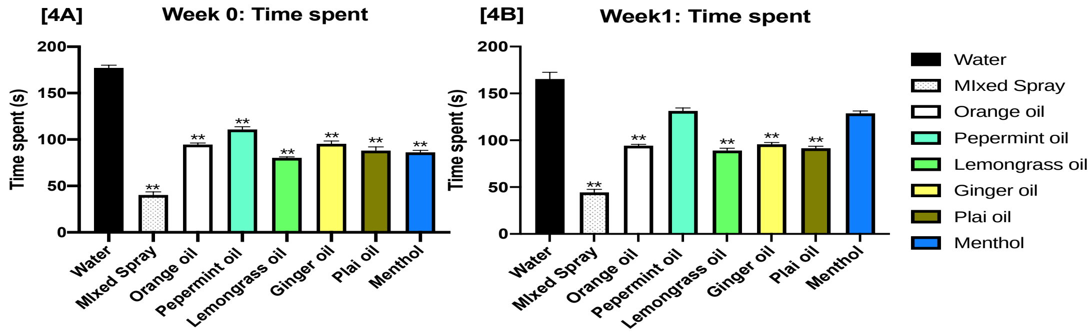

Figure 4. The time spent in each arm. The results were expressed as the mean ± SEM (n =10-12). Week 0 is presented in Panel A, whereas week 1 is presented in Panel B. ** $P < 0.001$ vs water.

Effect of herbal oils alone and in combination in the light/dark transition test

The light/dark transition test is based on fasting induced anxiety-like behavior. An increase in the amount of time spent in the light chamber, away from the repellent test substances in the dark chamber, indicates a mouse repellent effect. For the short-term effect (Figure 5A), the mice spent considerably more time in the light chamber for each repellent test substance compared to water. The combination spray showed the highest repellent effect followed by lemongrass oil, peppermint oil, ginger oil, plai oil, orange oil, and menthol. After one week (Figure 5B), only the mixed spray, lemongrass oil, ginger oil, plai oil, and orange oil retained their repellent effect.

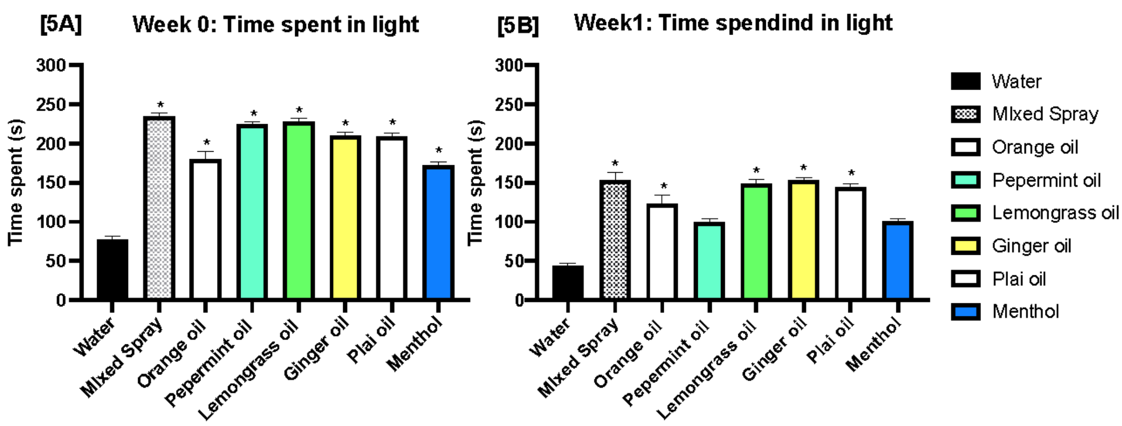

Figure 5. The time spent in light chamber. The results were expressed as the mean ± SEM (n =10-12). Week 0 is presented in Panel A, whereas week 1 is presented in Panel B. * $P < 0.05$ vs water.

In addition, the time spent in the darkened chamber were recorded. In terms of acute effect, the combination spray and all herbal oils spent significantly less time in the darkened chamber when compared to water. One week later, the combination spray and all herbal oils, with the exception of peppermint oil and menthol, spent significantly less time in the darkened chamber than water (Figure 6B).

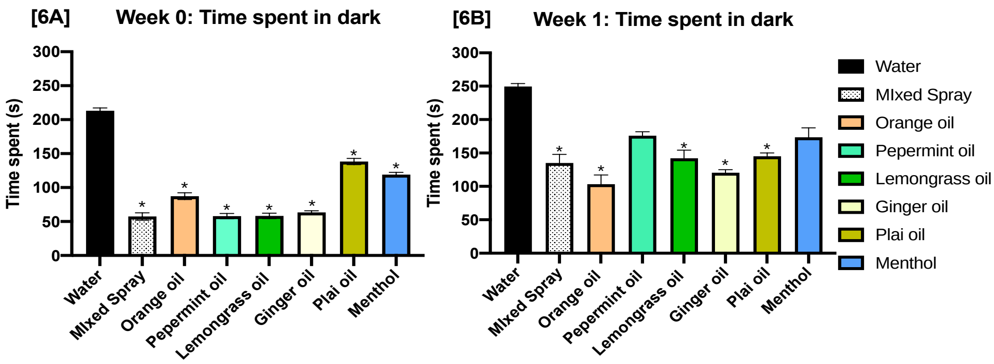

Figure 6. The time spent in dark chamber. The results were expressed as the mean ± SEM (n =10-12). Week 0 is presented in Panel A, whereas week 1 is presented in Panel B. * $P < 0.05$ vs water.

Locomotor activity test

To reduce the possibility of false positives in the radial arm test arising from increased locomotor activity through behavioral disinhibition (Beniger et al., 2009), the number of movements made by mice was used to assess their locomotor activity. There was no significant difference in the total number of arm entries in five minutes across experimental groups indicating no effect on locomotor activity (Figure 7).

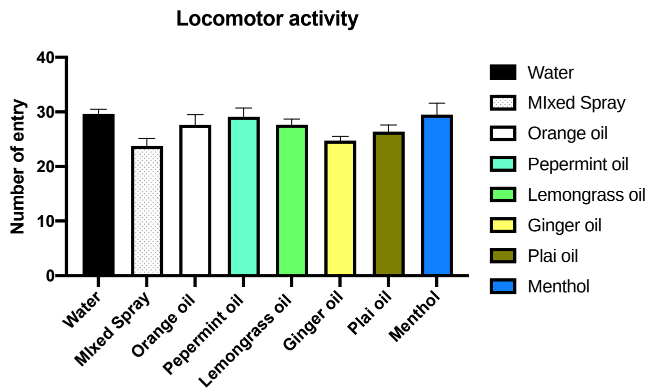

Figure 7. The effect of the single herbal sprays and its combination on locomotor activity of mice repellent test using radial 8-arm maze. The results were expressed as the mean ± SEM (n = 10-12). There was no significant difference among experimental groups.

DISCUSSION

The common house mouse can cause significant damage in the home as well as present a hazard to human health. Many methods have been employed to control the house mouse including rodenticides, trapping, and repellents (Jokić et al., 2017). However, these chemical products are often toxic and can exert their own negative impacts on human health and the environment. Herbal based products are an attractive alternative to these chemicals because they are rapidly degraded, have low toxicity and exhibit low potential for developing resistance (Bari et al., 2020). It is well recognized that phytochemicals from aromatic plants can have rodent repellent activity (Beninger et al., 2009) including bergamot oil, lavender oil, peppermint oil and eucalyptus oil (Singla et al., 2014; Jokić et al., 2017). In this study, behavioral models (radial arm maze and light/dark transition test) were used to evaluate the mouse repellent activity of orange oil (*Citrus sinensis* (L.) Osbeck), peppermint oil (*Mentha* × *piperita* L.), lemongrass oil (*Cymbopogon citratus* (DC.) Stapf), ginger oil (*Zingiber officinale* Roscoe), plai oil (*Zingiber cassumunar* Roxb.), menthol and a combination spray.

The radial arm maze has typically been used to assess rodent spatial memory, but this maze can also be used to assess potential attractants or repellents based on the natural behavior of rodents and their sense of smell, touch and taste (Witmer et al., 2014). Latency time and time spent in an individual arm indicates a substances repellent activity (Bari et al., 2020). On week 0, referring to the acute or short-term effect, we found that the combination spray and all herbal oils repelled the mice as shown by significantly longer latency times and shorter times spent in individual arms compared to water. After seven days, referring to the long-term effect, the combination, lemongrass oil, orange oil, plai oil and ginger oil retained their repellant activity whereas peppermint oil and menthol failed to repel the mice. The light/dark transition test is regularly used for measuring anxiety-like behavior in mice and is based on the natural desire of mice to spend more time in a dark area (Khamphukdee et al., 2017). Before the test, anxiety was induced in the mice by fasting for 18 hrs. The repellent substance was placed in the dark area with the mice and the total time spent in the bright and dark chambers were recorded. If the repellent was effective then the time the mice spent in the dark chamber with the repellent would be lower than the time spent in the light chamber (Grafe and Bhatnagar, 2018). On week 0 we found that mice exposed to the combination spray or any of the herbal oils spent significantly less time in the dark chamber (and more time in the light chamber) compared to mice exposed to water, indicating that all substances had mouse repellant activity. After seven days, the combination, lemongrass oil, orange oil, plai oil and ginger oil retained their repellant activity, but peppermint oil and menthol did not. The number of entries into each arm of the radial arm maze was counted to evaluate locomotor activity and rule out false positive results. This showed that water and the other repellent substances did not affect locomotor activity in the behavioral test. Our findings provide scientific confirmation of the local wisdom that orange oil, peppermint oil, lemongrass oil, ginger oil, plai oil, and menthol repel mice. Although herbal-based products have been reported to have low toxicity due to their rapid degradation (Bari et al., 2020). Nevertheless, their effects on humans should also be clarified.

Previous studies have reported on the rodent repellent properties of herbs based on their essential oils and terpenoids (Singla et al., 2014; Sharifi-Rad et al., 2017). Limonene is a terpenoid found in lemongrass (*Cymbopogon citratus* (DC.) Stapf) and orange (*Citrus Sinensis* L.) and has been reported as a cat, dog and insect repellent (Yoon et al., 2009). Ginger oil (*Zingiber officinale* Roxb.) and plai oil (*Zingiber cassumunar* Roxb.) both belong to genus *Zingiber* and both of these plants contain essential oils with antimicrobial, anti-inflammatory and repellent activities (Sharifi-Rad et al., 2017). However, phytochemical analysis of these plants should be conducted to identify the substances responsible for rodent repellent activity.

Menthol is a major compound found in peppermint (*Mentha* × *piperita* L.) and can be isolated from *Mentha canadensis* L. (cornmint) (Brahmi et al., 2017). Menthol has been used as a rodent repellant because of its strong odor and cold effect on the menthol receptor, which is expressed in a subset of mammalian sensory neurons by the transient receptor potential melastatin 8 (TRPM8) (Johnson et al., 2009). From our results, peppermint oil and menthol showed no significant repellent effect after 1 week. This may be caused by the repellent substance in menthol and peppermint losing its cold effect. As a result of these findings, it appears that herbs can be effective repellents and spraying should be done at least once a week, while the commercial herbal-based mice repellent products suggest that it needs to be repeated every 5 days, and then every week. That is considerably more expensive than our study in terms of economic assessment. Thus, it is economically justified to apply herbal oil or its combination as a mouse repellent. However, the efficacy should be evaluated in comparison to commercial products.

CONCLUSION

The mouse repellent activity of herbs alone and in combination was assessed using the radial arm maze and light/dark transition test behavioral models. The combination spray showed the strongest repellent activity and might be suitable for development as an alternative to synthetic substances.

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AUTHOR CONTRIBUTIONS

Yaowred Chulikhit controlled experiments. Orawan Monthakantirat and Supawadee Daodee assisted to design experiments and proofed the data. Juthamart Maneenet assisted in conducting the experiments. Charinya Khamphukdee analyzed data and performed the statistical analysis and data visualization and wrote the manuscript. All authors have read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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