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Mosquito Repellency Effects of The Essential Oils from *Cinnamomum iners* Leaves and Barks

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Abstract. Synthetic mosquito repellents containing DEET (N, N-diethyl-3-methylbenzamide), DEPA (N, N-diethyl phenylacetamide), permethrin and deltamethrin as active components are known to be the most effective repellents. However, these types of repellents have contributed to numerous toxic effects on not only the environment, but also non-target organisms. The use of natural products such as essential oils is reported as a safe alternative. To date, the study on repellency activities against mosquitoes using Malaysian plants is rare. In the present study, essential oils from leaves and barks of *Cinnamomum iners* have been reported to show repellency activity, and the whole plant have been used by the local communities to repel mosquitoes and other blood-sucking insects. Repellency assay of the oils against *Aedes aegypti* was conducted using the percentage repellency bioassay method at three different concentrations: 1%, 5% and 10%. The results showed that the essential oils from barks had a significant percentage repellency ($p < 0.05$) when compared to essential oils from leaves. Two main compounds were identified using the GC-MS: 1-isopropenyl-4-methyl-1,2-cyclohexanediol and 2-octen-1-ol,3,7-dimethyl (geraniol) from the bark and leaf essential oils, respectively. The essential oils demonstrated promising insect repellency activity which has the potential for further development into formulations that may serve as alternatives to DEET or be used as natural bioinsecticides to kill mosquitoes.

1. Introduction

An estimated 3.3 billion people around the world are at risk of contracting malaria caused by parasites such as *Plasmodium malariae* Feletti and Grassi, and *Plasmodium ovale* Stephens while almost 2.5 billion people are threatened by dengue haemorrhagic fever caused by its vector, *Aedes aegypti*. Furthermore, a mosquito-vector disease called filariasis or elephantiasis threatens about 1 billion people in 80 countries [1]. Synthetic chemicals have been developed in order to protect human from mosquito bites, being DEET (N,N-diethyl-m-toluamide) not only a broad spectrum repellent, but also the most effective and persistent on skin [2]. Even though the use of synthetic repellents generally tend to be more effective and/or longer lasting to control insects and arthropods compared to natural products [3,4], the use of chemicals raises several concerns related to environment and human health. In addition, several studies have shown that continuous application of synthetic organic insecticides can give rise to the development of resistant



populations of *Ae. aegypti* [5-7]. These concerns have alerted the scientific community to the urgent need of seeking alternative technologies for vector control.

Due to the increasing reported cases caused by mosquitoes, this study aims to evaluate a natural resource that has the potential to be developed as a safe, affordable, yet significantly effective material to repel mosquitoes. One of the promising approaches is using plant essential oils as the natural repellents. Numerous essential oils extracted from genus such as *Cymbopogon* spp., *Eucalyptus* spp. and *Ocimum* spp. have been shown to have high repellency against arthropod species and also well documented. Essential oils (EO) are complex mixtures of volatile organic compounds, constituted by hydrocarbons and oxygenated compounds, frequently are the ones that responsible for the distinctive odor of plants [8].

Cinnamomum iners Reinw. ex Blume is one of the 250 species from the genus *Cinnamomum* of the family Lauraceae, and commonly known as wild cinnamon or “pokok teja”. The leaves are widely utilized as a traditional medicine to relieve fever and digestive system problems, and as carminative [9], analgesic and antipyretic material [10]. Daily use of the bath water steeped with the boiled leaves can be effective in treating rheumatism. In addition to that, consuming the leaf juice cures headache and fever, and applying crushed leaves on minor wounds can heal the affected area [11]. The leaves have also been used extensively in joss sticks and mosquito coils due to their fragrant nature and high mucilage content [12]. The non-medicinal usage of the plant leaves includes raw materials in food and industrial products such as plastic, gum, paper, tyre and glass fibre [13].

The bark of *C. iners* has multiple traditional uses, containing some essential oils. The bark is used as a detoxifying agent when consumed as herbal tea. The bark sold as “mesni” in Malaysia is used as medicinal tea and curry flavour. A decoction of the bark is drunk after giving birth as part of the post-partum medicine. A decoction of the roots is drunk to cure fever, and very popular in the post-partum treatment to regain energy, and to improve blood flows and womb contraction. Meanwhile, the wood is used to build houses, household cabinet and furniture [11, 13]. However, it yet lacks studies on its ability to repel insects, such as mosquitoes. It has been communicated verbally by the local Chinese community that burning the barks would repel mosquitoes and houseflies [14].

Several phytochemical analysis showed that constituents such as linalool, caryophyllene oxide, and cardinol were identified from the leaf essential oils [15], and 1, 8-cineole, α -terpineol, terpinen-4-ol, β -pinene and caryophyllene oxide were identified from the stem bark essential oils [14,16]. Its extract was found to contain tannins [17], cardiac glycoside, flavonoid, polyphenol, saponin, sugar, and terpenoid [18, 19].

Another study [18] reported the presence of α -caryophyllene, stigmasterol, cardiac glycoside, flavonoid, polyphenol, saponin, sugar, tannin and terpenoid in the tree [20]. The latest study [20] using the barks and leaves of *C. iners* collected from Guimaras, Philippines showed the presence of seven compounds in the dichloromethane extract. After several series of chromatography of the bark oils, the isolated compounds were identified as 5,7-dimethoxy-3c,4c-methylenedioxyflavan-3-ol and β -sitosterol, 4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one, cinnamaldehyde, linoleic acid, and vanillin. The leaves of *C. iners* contained eugenol, linoleic acid, and β -sitosterol. For the mosquito repellency activity, citronellal, azadirachtin, linalool and p-menthane-3,8-diol were identified as the active constituents responsible for the activity, other than citronellol, geraniol and citronellic acid [21].

2. Materials and Methods

2.1 Plant materials

The barks and leaves of *C. iners* were collected in Machang district, Kelantan, Malaysia. The plant was verified by a botanist from the Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), and its voucher specimen (UKMB40385) was deposited to the UKM's herbarium.

2.2 Extraction of the essential oils

Air-dried and ground leaves and stem barks of *C. iners* (two batches of about 25 g) were subjected to hydrodistillation with 200 ml of distilled water for 3 hours using the original Clevenger-type apparatus. During the distillation process, the oils were dissolved in the distillation and called essential oils. The dissolved oils were recovered using hexane as an extractant with 10:1 (hydrosol: hexane) proportion, and were shaken gently for 30 minutes to produce two layers: water and hexane. The moisture from the hydrosol was removed using anhydrous sodium sulphate, and measured. The essential oils trapped in hexane were separated using a rotary evaporator to obtain secondary extracts and were analyzed immediately. The hydrosol later was exposed to a vacuum environment at room temperature for a short period of time to eliminate the solvent completely and was measured on an analytical balance and gravimetric to ensure that all of the solvents had evaporated.

2.3 Mosquito Repellency Activity

Due to the ethical considerations and the uncertainty that the insects used were not infected with known human pathogens, this study did not use humans as volunteer subjects. Besides that, it also included sensitization reactions following the tick, flea or mite bites. Therefore, the mosquito repellency assay was conducted using the percentage repellency bioassay method with slight modifications [22].

Three hundred *A. aegypti* eggs were supplied by Mr. Ariffin Majid from the Institute for Medical Research (IMR), Malaysia. Test solutions of 1%, 5% and 10% concentration which were dissolved in ethanol, have been used to evaluate the repellency activity. Then, 0.5 ml of the solution was applied onto one half of a round filter paper (22.7 mm diameter) and allowed to dry before testing. The filter paper was then placed inside a 100 ml measuring cylinder, and the cylinder was placed horizontally. Approximately ten adult female mosquitoes were introduced to the glass cylinder, one at a time. The timing began 1 minute after the introduction of the mosquitoes. The distribution of the mosquitoes inside the air-static test apparatus was observed over a 30-minute period for each treatment. The distribution, and number of individuals on the treated and untreated side were recorded in 10, 20 and 30 minutes. The experiments were repeated in triplicates. The percentage of repellency was calculated as follows:

$$\text{Repellency Percentage} = \frac{\text{Number of individuals on untreated half} - \text{number of individual on treated half}}{10} \times 100$$

The positive control of the mosquito repellent was tested by using the natural mosquito repellent, *Citronella* essential oil. The essential oil was prepared by using the same extraction method with *C. iners* essential oil. The control was tested by using the percentage repellency bioassay method. For the positive control, 0.5 ml of the essential oil was applied to the tested materials for each trial and the assay was repeated in triplicates.

2.4 Gas Chromatography Mass Spectrometer (GC-MS) Analysis

To identify the constituents occurring in the plants, the samples were subjected to GC-MS. The analysis was performed on a 7890 gas chromatograph (Agilent Technology) equipped with a 5975 mass

spectrometer (Agilent Technology). A fused silica capillary (Agilent Technology) HP-5MS UI (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., film thickness of 0.25 μm) was used for the separation. The injector temperature was set at 150 °C, and the detector temperature was set at 250 °C. During the first 4 minutes, the temperature was kept at 100 °C and the temperature was gradually increased to 130 °C at the rate of 5 °C/min and was held for 20 minutes. The compounds were identified by comparing their recorded mass spectra with the standard mass spectra from libraries provided by the GC-MS software system, literature data and the main components standards. Two injections from each sample underwent quantitative analyses by measuring the peak area normalization and calculating them as a mean value.

2.5 Statistical analysis

All data were collected in triplicates and expressed as mean ± SD. Statistical Package for Social Science (SPSS) version 23 was used to analyse the data. The significant differences were determined using one-way analysis of variance (ANOVA), with $p < 0.05$ being considered as statistically significant.

3. Results and Discussion

3.1 Mosquito Repellency of *C. iners* oils

The essential oils from the barks of *C. iners* exhibited a relationship between the concentrations tested, as well as a significant repellency activity (0.000; $p < 0.05$), with a repellency percentage of $9.30 \pm 3.91\%$ after 10, 20 and 30 minutes of observation. The repellency percentage for the 5% concentration was significantly highest at $12.33 \pm 1.41\%$, followed by the 10% concentration at $10.33 \pm 1.73\%$ while the 1% concentration showed the lowest repellency percentage at $5.22 \pm 3.80\%$. Furthermore, the repellency percentage of all three concentrations decreased substantially after 20 to 30 minutes (Figure 1A).

On the other hand, the leaf oil showed no relationship between the concentrations tested, as well as no significant repellency activity (0.314; $p > 0.05$), with a repellency percentage of $9.37 \pm 3.47\%$ after 10, 20 and 30 minutes of observation. The repellency percentage showed that the 1% concentration had the highest percentage of repellency at $10.78 \pm 2.54\%$ compared to the 5% and 10% concentrations at $9.00 \pm 2.83\%$ and $8.33 \pm 4.58\%$, respectively. Although the concentration of 1% showed the highest repellency percentage, the concentration of 10% was predicted to have longer repellence effect based on the steep increase after 20 minutes of exposure (Figure 1B). Comparing the repellency activities between the leaf and bark oil with *Citronella* oil (control), both leaf and bark oils showed significant mosquito repellency with a percentage of $10.85 \pm 2.41\%$. In addition to that, both oils showed significant mosquito repellency activity (Figure 1C).

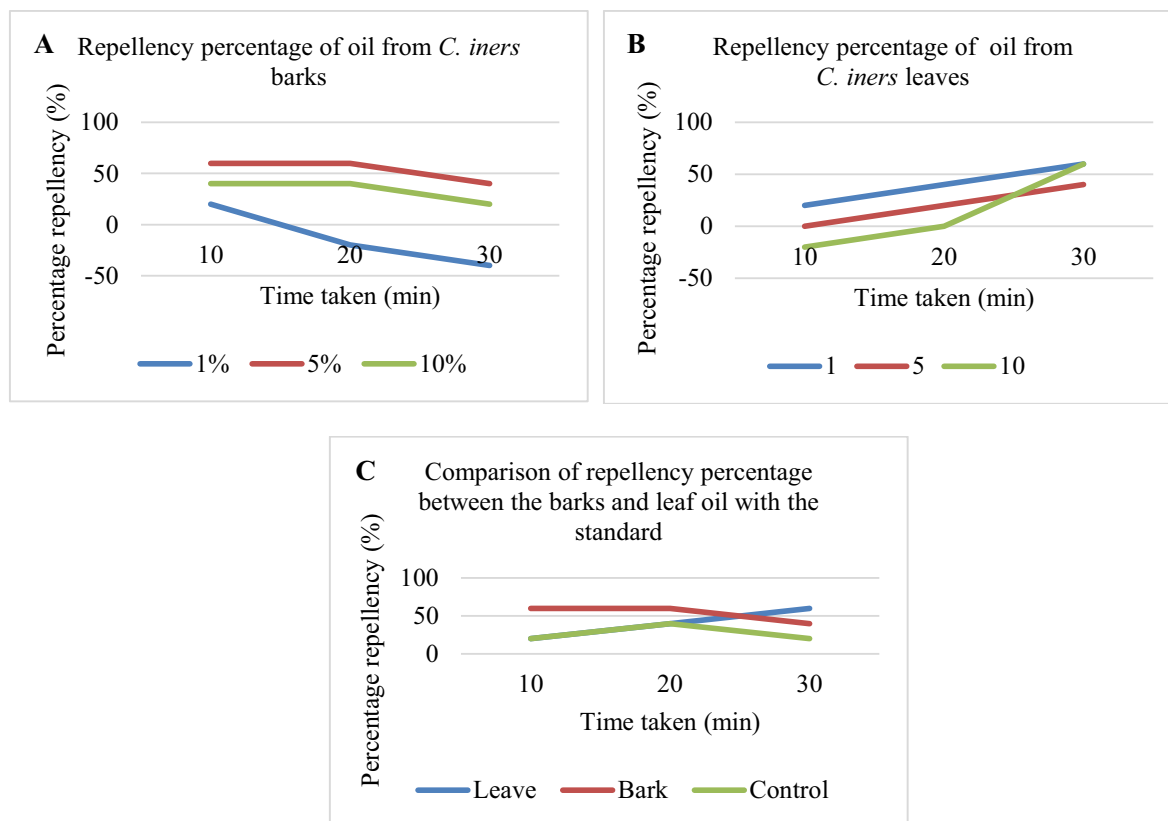


Figure 1. Percentage of mosquito repellency using *C. iners* (A) bark and (B) leaf essential oils, and (C) compared with *Citronella* oil (standard)

The results agreed to that by [23] who reported that the *C. iners* oil exhibited promising antifungal, antiviral, antibacterial and larvicidal activities. The repellency of oils from *C. cassia* bark was at 91% when tested at a dose of 0.1 mg/cm² while the *C. camphora* steam distillate repelled *A. aegypti* at 94%, which was comparable to DEET (82%) [24]. The duration of the effectiveness for oils from *C. cassia* bark was comparable to DEET, lasting for approximately 1 hour while a relatively short duration of repellency was observed in *C. camphora* steam distillate. Furthermore, [25] reported the insecticidal properties of another species of *Cinnamomum*, *C. osmophloeum* essential oils. The test demonstrated that the leaf essential oils had an excellent inhibitory effect against the larvae of *A. aegypti*. The LC₅₀ values for the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type against the *A. aegypti* larvae in 24 hours were 36 ppm and 44 ppm, respectively.

The woods of *C. zeylancum* dose-dependent KD₅₀ and KD₉₀ values were 12534 mm and 16065 mm, respectively while its mortality value was 84+1%. In addition to that, the woods of *C. jauamcum* showed similar trend with its KD₅₀ and KD₉₀ value of 12922 mm and 16721 mm, respectively while its mortality value was 89+3% [26]. In another study, the leaf oil of *C. mollissimum* showed a significant repellency effect with the ED₅₀ ranging from 0.0023 to 0.0065 mg/cm². Furthermore, when the oils were incorporated in an insect repellent cream formulated with other ingredients, they showed 96.6% protection against *A. aegypti* mosquito bites for the duration of the test [27].

The leaf oil from *C. aromaticum* did not repel adult *Blattella germanica*, moderately repelled *Blatta orientalis*, and exhibited good repellency to *Cochliomyia hominivorax*. Meanwhile, the formosan *C. camphora* oil did not repel *Lucilia cuprina* while the leaf oil of *C. tamala* repelled *Apis florea* bees, and the bark and leaf oil of *C. verum* repelled *Blattella germanica*. The cinnamon oil was considered to be one of the better repellent against *Cochliomyia hominivorax*. The findings from this study showed that the essential oils extracted from the plant had the potential repellency activity against mosquito larvae [27].

3.2 Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

Further analysis was conducted to identify constituents that possibly contributed to the repellency and toxicity activities. Both bark and leaf oils were analysed using the gas chromatography-mass spectrometry (GC-MS). The results showed the presence of (1S-(1 α ,2 α ,4 β))-1-isopropenyl-4-methyl-1,2-cyclohexanediol in the bark oil (Figure 2), and 2-Octen-1-ol, 3,7-dimethyl (geraniol) in the leaf oil (Figure 3).

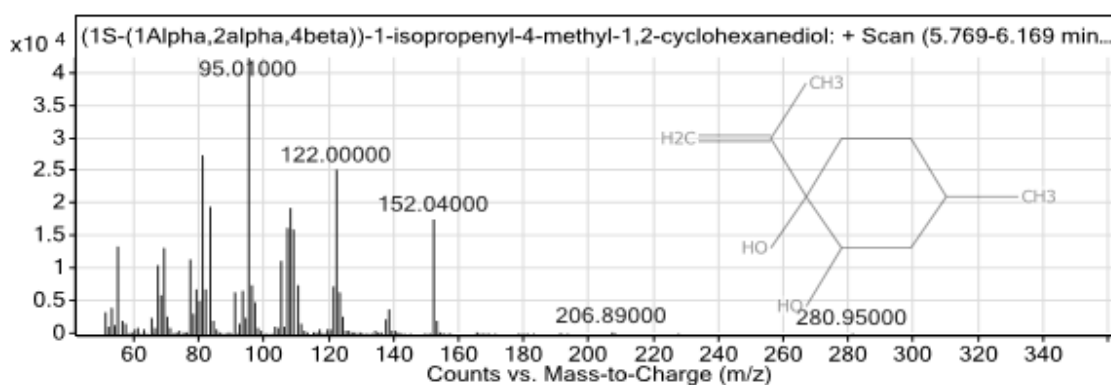


Figure 2. Major compound, 1-Isopropenyl-4-methyl-1,2-cyclohexanediol was identified in the bark oil of *C. iners*

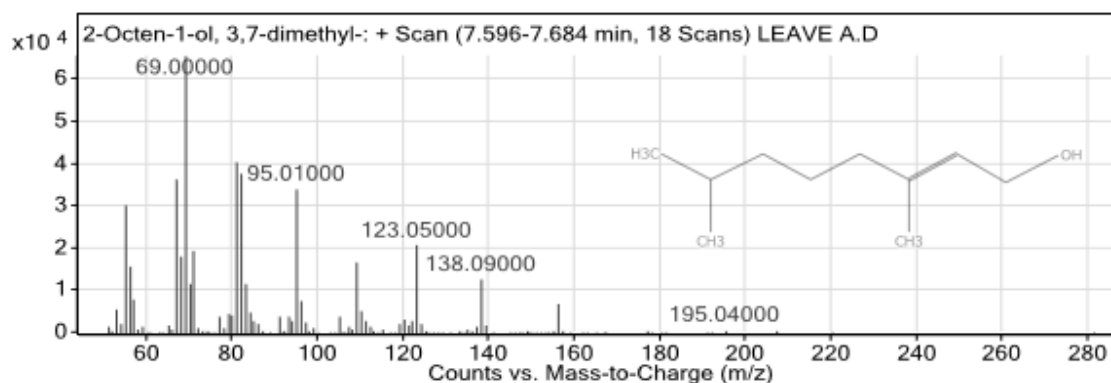


Figure 3. Major compound, 2-Octen-1-ol, 3,7-dimethyl (geraniol) was identified in the leaf oil of *C. iners*

In this study, compounds found in the bark oil were 1-isopropenyl-4-methyl-1,2-cyclohexanediol (100%) and 9,12,15-octadecatrienoic acid (linoleic acid) (5.59%), while 2-octen-1-ol,3,7-dimethyl

(geraniol) (100%) and 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl (54.37%) were identified from the leaf oil. Geraniol, which is also called rhodinol, is a monoterpene and an alcohol, exhibiting a pleasant rose-like odour [28]. Geraniol together with other oxygenated compounds isolated from the essential oil of *Dianthus caryophyllum*, showed strong repellent activities against ticks [29]. In addition, geraniol identified in 12 Kenyan plants found to be one of the most effective repelling chemicals against *A. gambiae* (Diptera) [30, 31].

Hydrocarbons particularly monoterpenes, as well as oxygenated compounds are major components of many essential oils and are the most important group presenting mosquito repellent activity [8]. This type of compounds contained in the *C. iners* oils could be considered for repelling insects [32]. Plants containing terpenes may be used as repellents without modification by rubbing fresh leaves onto the skin to release the oils; they may also be bruised to release the oils and then hung around the house. Other uses may be as fumigants when the fresh leaves are burned or the oils are volatilized. They are also commonly added to commercial insect repellents labelled as “natural”. Many terpenes are volatiles; consequently, their vaporous form deters phytophagous insects by affecting their olfactory receptors [32].

Comparing with the previous phytochemical analysis, the leaf oil of *C. iners* contains major bioactive compounds: linalool (35-50%) and benzyl benzoate (32.7%) [12]; cinnamic aldehyde, 2-hydroxycinnamaldehyde, cinnamophilin, caryophyllene, caryophyllene oxide, hydroxychalcone, coumarine eugenol, safrole, geraniol, xanthorrhizol, and camphour [11,33]. Meanwhile, the bark oil has been reported to contain 1,8-cineole as the major compound (40.75%), β -pinene, α -pinene, myrcene, α -phellandrene, α -terpineol, terpene-4-ol, γ -terpinolene, linalool, and caryophyllene oxide. The wood oil of *C. iners* contains mainly sesquiterpenes (57.6%) of which eudesmol (24.4%) and guaiazulene (18.2%) are found in greatest quantity [34]. The root oil contains saponin, terpene, eugenol, cinnamic aldehyde, benzaldehyde, and safrole [11].

A study by [35] was also in agreement with the other reports, stating that the *C. iners* leaves contained low alkaloid but high terpenoid (β -caryophyllene), α -pinene (0.15%), eucalyptol (1.17%) and limonene (0.11%). Furthermore, [25] reported that the chemical compositions of leaf essential oils from eight provenances of indigenous cinnamon (*C. osmophloeum*) were classified into five chemotypes: cinnamaldehyde type, linalool type, camphor type, cinnamaldehyde/cinnamyl acetate type, and mixed type.

The compounds, 1-isopropenyl-4-methyl-1,2-cyclohexanediol and 2,6,10-dodecatrien-1-ol,3,7,11-trimethyl, were reported for the first time in the bark and leaf oil of *C. iners*. The different content of the oils extracted in the present study could be due to the different method of extractions applied as stated by [36]. Factors such as the pre-extraction preparation method, size of the samples, type of soil, temperature, light and humidity of surrounding area, and environmental conditions affect the yield as well as compounds in the oils. In this study, the secondary or recovery oils were used to determine the factors that could cause the loss of compounds, and limit the findings.

The repellency effect of the essential oils indicated that they contained active components responsible for the activity. The major components of the oils, when in combination with other compounds of diverse structures in the oils, could exhibit different mode of actions, contributing towards their repellency activity. According to [32], different results shown by various studies were contributed by various factors, such as mosquito taxonomy and genetics, individual human-subject differences, conditions of use and formulation chemistry that affects the repellency performance.

4. Conclusion

The essential oils extracted from *C. iners* barks significantly repelled the mosquitos. Terpenes contained in the oils were probably associated with this significant activity. The study could be extended for further

development into formulations that may serve as alternatives to DEET or be used as a natural bioinsecticide which is safe to humans, efficient and affordable to kill mosquitoes.

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