

## Chemical Composition of *Cinnamomum Cassia* Oil in Vietnam and Its Spatial Repellent Effects Against the *Aedes Aegypti*

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ARTICLE INFO		ABSTRACT
Received:	14/3/2022	Dengue is a mosquito-borne viral disease that has caused millions of dengue virus infections per year in the world. Mosquito repellent is an effective way to prevent mosquito bites and the spread of mosquito-borne disease. In this study, we extracted essential oil from the bark of <i>Cinnamomum cassia</i> collected in Viet Nam and analyzed its chemical composition. We then evaluated the spatial repellent of the essential oil basing on WHO guidelines. The essential oil was hydro-distilled and extracted with chloroform to obtained an average yield of 2.55% (w/w). The main component of oil is <i>trans</i> -cinnamaldehyde with a content of 99.24%. The repellent index of essential oil at concentrations of 25%, 15%, 5%, 1% and DEET 10% after 90 minutes was 73.33%, 57.14%, 33.33%, 17.65% and 12.50%, respectively. In conclusion, the <i>Cinnamomum cassia</i> essential oil is an effective and promising repellent in preventing dengue mosquitoes.
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### 1. Introduction

Dengue is a mosquito-borne viral disease that has caused about 390 million dengue virus infections per year, of which 96 million manifest clinically [1]. The primary vectors that transmit the disease are *Aedes aegypti* mosquitoes. There has been no specific treatments for dengue/severe dengue. Dengue prevention and control depend on effective vector control measures including the use of insecticide to reduce the mosquito population and mosquito repellents to protect humans from mosquito bites.

Mosquito repellent is an effective method to prevent mosquito bites. There are two kinds of repellents, which are contact and spatial repellents. Contact repellents are applied on skin to prevent mosquito bites. These popular contact repellents compose of DEET, picaridin, which have low volatility and can remain on the surface for a long time to repel mosquitoes. However, contact repellents can be absorbed into the skin, and people need to re-apply them frequently to keep their effectiveness. Spatial repellent provides an alternative. In general, spatial repellent is easy to volatile and diffuse through an open area. Currently, both botanical and synthetic spatial repellents are used in the market.

Although synthetic repellents tend to be more effective and/or longer-lasting than botanical repellents [2], concerns have been raised over the safety of these repellents to the human being as well as potential toxicity to the ecosystem and the resistance to synthetic repellents [3] - [4]. Recent studies have found essential oils as potential candidates for mosquito repellents. The natural oils are considered safe and environmentally friendly. They also cause less resistance to the mosquito.

Vietnam is a country with a high incidence of dengue fever every year. Vietnam has many natural plants that can be a potential source for use as a mosquito repellent. In this study, we extract essential oil from *Cinnamomum cassia* from Northern Vietnam, and evaluate mosquito spatial repellent of the essential oil to *Aedes aegypti* mosquito.

## 2. Materials and Methods

### 2.1 Essential oil extraction

*C. cassia* bark was purchased from a Moc Yen Bag company in Tran Yen district, Yen Bai province in Northern Vietnam. For each experiment, 30 g of *C. cassia* sample was cut into small pieces and immersed into distilled water with a ratio of 10:1 (v/w, mL/g). The mixture was extracted by hydrodistillation in a Clevenger-type apparatus. 150 mL whitish distillate was collected as an emulsion of *C. cassia* essential oil (EO). Chloroform (50 × 3 mL) was used to extract essential oil from the water phase three times. The organic fraction was dehydrated over anhydrous sodium sulfate and filtered. The solvent is then completely removed by evaporation in a rotary evaporator, the resulting volatile extract was kept at 4 °C for further analysis. The percentage of essential oil yield was calculated as the weight of essential oils divided by the weight of bark powder.

### 2.2 GC/MS analysis

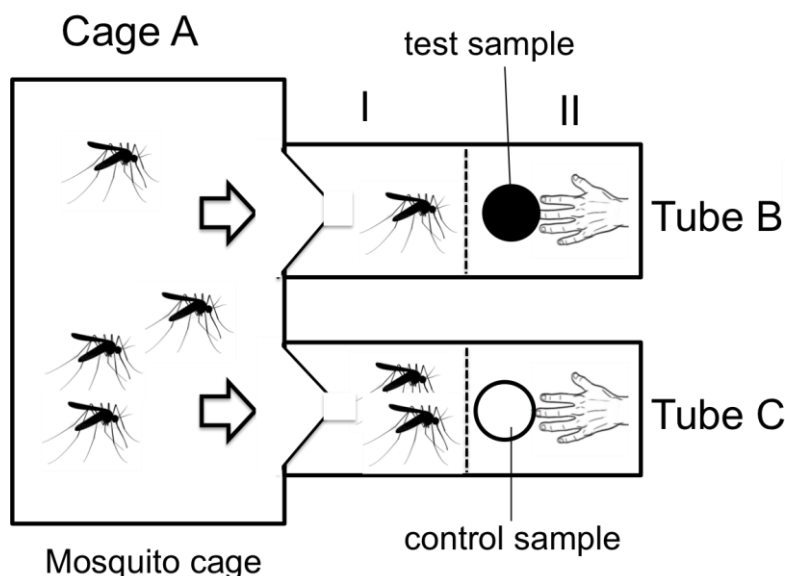
The essential oil was analyzed as our previous report [5]. Briefly, the essential oil was analyzed by Gas chromatography-mass spectrometry system with Agilent 6890N gas chromatograph instrument equipped with an Agilent 5973 mass spectrometer and an HP-5MS capillary column (length 30 m × 0.25 mm ID, film thickness 0.25 mm; Agilent-Technologies, Palo Alto, CA, USA). Helium was used as the carrier gas with a constant flow of 1.0 mL/min. The temperature of the oven was set from 50 °C (held for 2 min) to 80 °C (2 °C/min), from 80 °C to 150 °C (5 °C/min), from 150 °C to 200 °C (10 °C/min), from 200 °C to 300 °C (20 °C/min) and held there for 5 min. The injector temperature was 250 °C. Hexane was used to dilute the samples with a ratio of 1:40 (v/v), then 1 µL of the diluted samples were injected in splitless mode. Components were identified based on MS library search (NIST). The percentage composition was calculated by integrating the peak areas of the chromatograms.

### 2.3 Mosquito rearing

The *Aedes aegypti* mosquitoes were reared at the Faculty of Pharmacy, Lac Hong University using the standard procedures described by Manh et al [5]. The insectary condition were kept at 27 ± 3°C, 70%–80% relative humidity. Larvae were reared in plastic trays and provided with fish food, whereas adult mosquitoes were kept in breeding cages (30 cm × 30 cm × 30 cm) and maintained on a 10% sucrose solution. The female mosquitoes were fed with blood of live mice for mosquito reproduction. The study was followed the Guide for the Care and Use of Laboratory Animals of Faculty of Pharmacy, Lac Hong University.

### 2.4 Spatial repellent evaluation

The system were modified from Y-olfactometer guided by WHO.[6] A scheme of this system is presented in Figure 1. This device was made from acrylic materials. The biting active mosquitoes will be released into Cage A (dimension of 40×30×30 mm), which is connected to the two tubes B and C, each containing either a test or control substance. The mosquitoes either remain in the cage or fly to be captured in one of the two tubes. Each tube has two parts. Part I is a trapping chamber with a length of 150 mm and a diameter of 90 mm. Mosquitoes enter this chamber and cannot return to Cage I (due to the funnel shape of Part I). Part II is a test/control chamber with a length of 200mm and a diameter of 90 mm. Part I and part II are separated by a net, therefore mosquitoes cannot fly from part I to part II. The volunteer will put their bare hands (left or right) in part II to serve as an attractant for mosquitoes. The hands are put near a filter paper (20-30 mm) that contains a sample test (essential oil) or a control blank sample (ethanol). The sample test was cinnamon oil diluted in ethanol at 1%, 5%, 15%, 25% (v/v) concentration or DEET 10%. For each test, 150 µl of one of the sample tests or 150 µl of ethanol control was applied to the paper that was placed on a small Petri. Then, 20 female active biting mosquitos are released into Cage A. The volunteer sat on a chair directly behind the tubes. The volunteer placed their hand in front of the Petri. In order to reduce inter-host variability, we used the same volunteer in all experiments. After 30, 60, and 90 minutes, the number of mosquitos were recorded in each tube. Each test was repeated three times.



**Figure 1.** Schematic drawing of the modified Y-tube olfactometer

The repellent index (RI) is the percentage of the difference of mosquitoes in the tube C (control sample) and the tube B (test sample) divided by the total number of mosquitoes in both tubes.

The repellency index (%) was calculated as equation (1):

$$RI(\%) = \frac{N_c - N_t}{N_c + N_t} \times 100 \quad (1)$$

$N_c$ : Total number of mosquitoes in tube C (control sample)

$N_t$ : Total number of mosquitoes in tube B (test sample)

The RI can be -100 to +100, with 0 is neutral response. If RI is positive, the substance will have repellent effects. If RI is negative, the substance will have attractant effects.

### 3. Results and Discussion

#### Yields and Chemical Constituents of the Essential Oil

The essential oil was obtained from the hydrodistillation of *C. cassia* bark and extracted by chloroform. The yield of the essential oil was 2.55% (w/w), calculated basing on the weight of the bark.

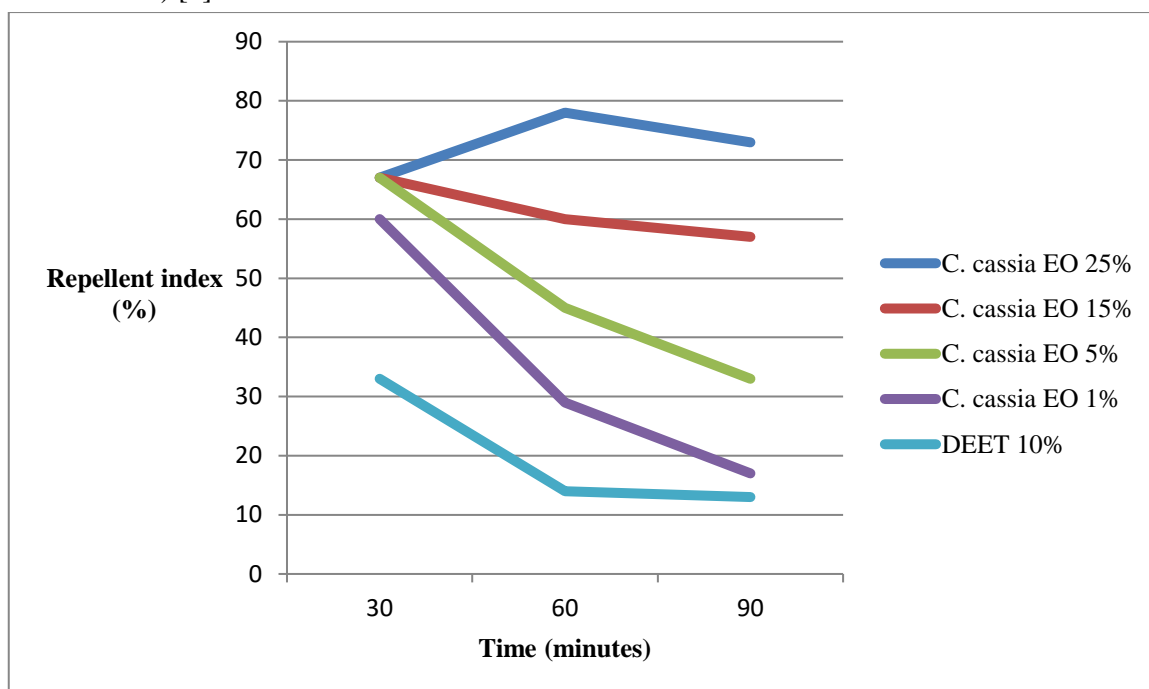
**Table 1.** Chemical constituents (%) of *C. cassia* essential oil.

No	RT (min)	Formula	Molecular mass	Compound	Percentage (%)
1	24.17	C <sub>9</sub> H <sub>8</sub> O	132	<i>trans</i> -Cinnamaldehyde	99.24
2	27.56	C <sub>15</sub> H <sub>24</sub>	204	$\alpha$ -Copaene	0.13
3	31.19	C <sub>15</sub> H <sub>24</sub>	204	$\alpha$ -Muurolene	0.21
4	31.77	C <sub>15</sub> H <sub>24</sub>	204	$\delta$ -Cadinene	0.37

RT: Retention times (minutes).

Table 1 shows the chemical composition of *C. cassia* essential oil. There are four components in the essential oil, of which the most abundant is *trans*-cinnamaldehyde (99.24%). The major component of *trans*-cinnamaldehyde was considered as the chemotype of *C. cassia* EO. Trinh et al.

reported similar results that the main component is 90% *trans*-cinnamaldehyde [7]. Similarly, Ito et al. showed that *C. cassia* essential oil in Yen bai contained a high percentage of *trans*-cinnamaldehyde ( $89.75 \pm 8.03\%$ ) [8].



**Figure 2.** Repellent index of *Cinnamomum cassia* essential oil in different concentrations

Figure 2 shows the repellent index of *C. cassia* EO in different concentrations. At 30 minutes, the repellent index of *C. cassia* EO at 5%, 15%, and 25% concentrations are similar, and their repellency index is higher than EO at 1% and DEET 10%. However, at 60 min and 90 min, the repellent index of *C. cassia* EO of 1%, 5%, and 15% show decreasing repellency. The lowest concentration EO of 1% shows the fastest decreasing repellency. The EO at 25% still shows good repellency until 90 minutes. The repellency index of DEET 10% is lower than *C. cassia* EO at all concentrations. This is because DEET is known as contact repellent, which means that DEET is effective when applied directly on human skin. DEET has lower volatility than *C. cassia* EO, therefore *C. cassia* shows better repellent in space. For a small space such as a room or a hut, this spatial repellent effect can reduce human–vector contact for a group of people rather than a single individual. It is important to note that *trans*-cinnamaldehyde is the major component in the *C. cassia* EO in this study. In a previous study by Chang et al., *trans*-cinnamaldehyde also show contact repellency against *Aedes aegypti* mosquitoes [9]. Mitra et al. 2019 showed that cinnamon oil is one of the best essential oils to reduce mosquito attraction to human odor [10]. Peach et al 2019 showed that a combination of cinnamon bark and lemongrass oil can enhanced the repellent effect of single EOs to *Aedes aegypti* mosquito through synergistic interactions [11].

#### 4. Conclusions

To our best knowledge, it is the first study to examine mosquito repellency of *C. cassia* essential oil from Vietnam. The main component of EO is *trans*-cinnamaldehyde. The spatial repellency of *C. cassia* EO is better at higher concentration, and better than DEET 10% in our assays. Future studies with larger human participants in a community are necessary to confirm the repellent efficacy of *C. cassia* essential oil.

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