Research paper

Repellent, larvicidal and adulticidal activities of essential oil from Dai medicinal plant Zingiber cassumunar against Aedes albopictus

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A B S T R A C T

Zingiber cassumunar is an important plant used in traditional medicine and as a natural mosquito repellent. However, the compounds responsible for the repellent activity of the plant are still unknown. The aim of the study is to identify the components of Z. cassumunar essential oil that show repellent activity against Aedes albopictus. We also evaluated the larvicidal and adulticidal activities of Z. cassumunar essential oil against Ae. albopictus. In-cage mosquito repellent experiments showed that Z. cassumunar essential oil possessed moderate repellent activity with a minimum effective dose (MED) of 0.16 ± 0.01 mg/cm², compared to reference standard N,N-diethyl-3-methylbenzamide (DEET, 0.03 ± 0.01 mg/cm²). Bioassay-guided fractionation identified the major active compound of Z. cassumunar essential oil as (−)-terpinen-4-ol (1) (MED: 0.19 ± 0.0 mg/cm²). We also found that Z. cassumunar essential oil showed moderate larvicidal activity against first instar larvae of Ae. albopictus with a LC50 of 0.01 mg/cm², compared to reference standard N,N-diethyl-3-methylbenzamide (DEET, 0.01 mg/cm²). Fumigation bioassays showed that Z. cassumunar essential oil has mosquito repellent activity, and that (−)-terpinen-4-ol is mainly responsible for this activity. Furthermore, this study provides scientific support for the folk usage of Z. cassumunar essential oil as mosquito repellent and indicates that Z. cassumunar essential oil and (−)-terpinen-4-ol can be used as plant-derived repellents and insecticides for mosquito control.

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1. Introduction

Mosquitoes, which are the main vector of mosquito-borne diseases such as dengue fever, yellow fever, malaria and chikungunya fever, seriously threaten human health (Brown and Hebert, 1997). The use of insecticides and repellents represent major strategies for preventing mosquito-borne diseases (Ma et al., 2019). Insecticides obtained by chemical synthesis are usually characterized by their high efficiency and speed. However, the widespread use of synthetic insecticides has increased environmental pollution and insect resistance (Ascher, 1993; Govindarajan et al., 2012; Raguraman and Singh, 1997). Insect repellents are an alternative strategy to control mosquitoes. The best-known repellent on the market is N, N-diethyl-3-methylbenzamide (DEET). However, studies have found that DEET produces a number of side effects, including urticaria syndrome, anaphylaxis, hypotension, and neurotoxicity (Adeiran and Fabiyi, 2012; Brown and Hebert, 1997; Corbel et al., 2009). By contrast, plant-derived repellents and insecticides, especially plant essential oils, have shown significant advantages such as rapid biodegradability, eco-friendliness, and safety for insect and mosquito control (Ali et al., 2015; Bedini et al., 2018; Wang et al., 2015; Yang et al., 2005). Therefore, it is essential to identify new plant-derived repellents and insecticides for mosquito control.
One potential source for plant-derived mosquito repellent is *Zingiber cassumunar* Roxb (Zingiberaceae), synonym of *Zingiber purpurcum* Roxsc., recorded in Flora of Yunnan, and commonly known as cassumunar ginger (*Kunming Institute of Botany, 1997*). *Z. cassumunar*, known in Thailand as *Zingiber montanum* (J.Koenig) Link ex A.Dietr (Sanatombi and Sanatombi, 2017; Verma et al., 2018) is called ‘bu lei’ by the Dai people living in Yunnan province of China, where it is an essential raw material in Dai medicine. One of China’s four major ethnic medicines. For example, it has been used as the main material of the Dai patent medicine called “pills of shuangjiang weitong” (Wei and Tang, 2018). The plant rhizome has been widely used in folk medicine to treat inflammations, sprains, rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wounds, asthma, coughs, skin diseases, dyspepsia, stomach bloating and also has the folk usage of rheumatism, muscular pain, wound...
compounds were confirmed by comparing their NMR data with those of previously reported data.

2.6. Bioassay-guided fractionation and purification of active compounds

*Zingiber cassumunar* essential oil (8.17 g) was subjected to a gradient elution of petroleum ether (PE)-ethyl acetate (EtOAc) (100:0, 50:1, 20:1, 1:1) on a silica gel column to yield four fractions: Fr.1, Fr.2, Fr.3 and Fr.4. Based on the repellent bioassay, Fr.2 (2.25 g) was further fractionated by a silica gel column, eluting with PE-CHCl₃ (10:1, 9:1, 8:1, 7:1) to successively give 1 ((−)-terpinen-4-ol, 435 mg) and 2 ((E)-1-(3,4-dimethoxyphenyl) butadiene, 330 mg).

2.7. In-cage mosquito repellent bioassay

We determined the minimum effective dosage (MED) of *Zingiber cassumunar* essential oil required for repellent activity using an in-cage mosquito repellent model described by Ma et al. (2019). These experiments were conducted at the Kunming Center for Disease Control and Prevention. Bioassays were performed using five concentrations of each tested sample (1.50, 0.75, 0.38, 0.19, 0.1, 0.05, 0.02 mg/cm²), which were prepared as follows. Tested sample including *Z. cassumunar* essential oil, fractions and compounds isolated from *Z. cassumunar* essential oil was dissolved with anhydrous ethanol to yield a 2 mL solution. Then, absorbent cotton gauze (5 cm × 10 cm) was treated with 1 mL of solution. The remaining 1 mL of solvent was diluted by adding 1 mL anhydrous ethanol and absorbent cotton gauze was treated with 1 mL of this solution. This process was repeated for three additional dilutions. The treated gauze was placed in a ventilated area for 3–5 min to allow the anhydrous ethanol to evaporate. Once dried, treated gauze was placed on the back of the hands of volunteers. First, the hands and arms of volunteers were wrapped in two layers of polyethylene film to protect them from mosquito bites. A window was cut in the plastic film (4 cm × 8 cm), which was covered by dry treated gauze. Volunteers placed treated hands into cages that contained three hundred 4- to 5-day-old adult mosquitoes. The number of mosquitoes that perched on the gauze was recorded during a 1-min test period. We regarded fewer than three bites as evidence of an effective dosage; fractions that showed repellent activity at high concentrations were subsequently tested at lower concentrations until the MED was determined. If three bites or more were recorded at a given concentration, a higher concentration was tested until a MED was determined. If a concentration of 1.5 mg/cm² was ineffective, the fraction was deemed ineffective. DEET was used as a positive control and ethanol was used as the solvent control.

2.8. Larvicidal bioassay

We evaluated the larvicidal activity of *Zingiber cassumunar* essential oil and its major constituents against *Aedes albopictus* by using the impregnation method described by Ma et al. (2019). Bioassays were performed using five concentrations of tested sample (104, 52, 26, 13, 6.5 µg/mL), which were prepared as follows. Briefly, 2.5 mg of each tested sample was dissolved in 15 µL dimethyl sulfoxide (DMSO) and diluted with deionized water to 12 mL sample solution. Then, 6 mL of sample solution was added to 0.6 mL of a 2% suspension of rat feed and was diluted with deionized water to 12 mL larval test solution. The remaining 6 mL sample solution was diluted by using the above dilution method, which was then repeated for three additional concentrations. We used an 8-cm Pasteur pipet to transfer first instar larvae (in 1 mL of test solution) to a 24-well plate. We placed five larvae in each well and used five wells. We then added 5 mL of larvae test solution to each well. We used DMSO (2.5 mg) as a blank control group, which consisted of five duplicate wells. Larval mortality was recorded after 24 h. Larvae that showed no movement in the well after manual disturbance of water by a pipet tip were recorded as dead.

2.9. Adulticidal bioassays

We evaluated the adulticidal activity of *Zingiber cassumunar* essential oil against *Aedes albopictus* by using airtight fumigation in conical flasks as described by Yang et al. (2005). Each 250 mL conical flask was placed into ten 4- to 5-day-old female mosquitoes. Filter paper (1 cm × 3 cm) was glued to the bottle of a rubber stopper. The rubber stopper was tightly stuffed into the conical flask to ensure a good seal. The test samples contained the *Z. cassumunar* essential oil, (−)-terpinen-4-ol, and (E)-1-(3,4-dimethoxyphenyl) butadiene. The *Z. cassumunar* essential oil was diluted with acetone to give five concentrations (1.6, 3.2, 6.4, 12.8 and 25.6%). The testing concentrations of (−)-terpinen-4-ol, and (E)-1-(3,4-dimethoxyphenyl) butadiene were 0.4, 0.8, 1.6, 3.2 and 6.4%. A volume of 10 µL of testing solution was immediately dropped on filter paper and the rubber stopper quickly stuffed into the conical flask. In the control groups, acetone (10 µL) was used to replace the test sample under the same conditions. Each sample was tested three times. The mosquitoes were constantly fumigated and the mortality was recorded at 0.5, 1, 2, 4, 8 and 24 h. Mosquitoes were considered dead when they did not move in response to flask shaking. The fumigating adulticidal activity of the testing samples against *Ae. albopictus* was evaluated by LC₅₀ (50% lethal concentration) and LT₅₀ (50% lethal time). LC₅₀ was obtained by the airtight fumigation model in conical flasks and tested under the concentration of the LC₅₀ at 0.5 h. The number of deaths was checked and recorded every minute for 30 min.

2.10. Statistical analysis

When mortality of the blank control was greater than 20%, larvicidal and adulticidal bioassays were discarded and repeated. LC₅₀, LC₉₀, LC₅₀, LT₅₀, 95% confidence intervals, and regression equation were analyzed to evaluate the larvicidal and adulticidal activities of *Zingiber cassumunar* essential oil against *Aedes albopictus*.

3. Results and discussion

3.1. Chemical composition of essential oil

Chemical constituents of essential oil from *Zingiber cassumunar* rhizomes were analyzed by GC-FID, GC–MS and NMR. Twenty-three components were identified, accounting for 92.75% of the overall composition. Sabinene (49.90%), (−)-terpinen-4-ol (13.51%, 1) and (E)-1-(3, 4-dimethoxyphenyl) butadiene (DMPBD, 10.31%, 2) were major compounds in *Z. cassumunar* essential oil (Table 1). *Z. cassumunar* essential oil was characterized by monoterpane hydrocarbons (63.88%), oxygenated monoterpenes (27.02%) and sesquiterpene hydrocarbons (1.85%).

Previous research on the chemical composition of *Zingiber cassumunar* essential oil from China, the identified sabi- nene, terpinen-4-ol, and γ-terpinen, but not DMPBD, likely because NIST 14 and retention index methods fail to identify this compound (Wang et al., 2015). In this study, DMPBD was isolated and identified by NMR. In addition, the optical rotation of (−)-terpinen-4-ol was firstly measured in present study with [α]₁₀⁰ = 15.88 (c 0.17, acetone). Terpinen-4-ol has been reported to exist as two optical isomers, which show different bioactivities in different plants (Li et al., 2015).
The chemical composition of the Zingiber cassumunar essential oil in various countries and regions has been reported. Previous studies (Bua-in and Paisooksantivatana, 2009; Leelarungrayub et al., 2017; Sukatta et al., 2009; Taroeno et al., 1991; Verma et al., 2018; Yinggam and Brantner, 2018) have shown that rhizome of Z. cassumunar essential oil from Thailand, India, and Indonesia are mainly composed of sabinene, terpinen-4-ol, and DMPBD, which is consistent with the results of our study. However, other studies have reported major components of Z. cassumunar essential oil that are inconsistent with our findings. For example, previous studies have reported that the main constituents of the Z. cassumunar essential oil from Thailand are sabinene, terpinen-4-ol, and γ-terpinen, respectively (Chaiyana and Paisooksantivatana, 2008). The chemical compositions of the Z. cassumunar essential oil from Malaysia and Bangladesh also differ from Z. cassumunar essential oil from China and Thailand; specifically, the main components of Z. cassumunar essential oil from Malaysia has been reported as 2,6,9,9-tetramethyl-2,6,10-cycloundecatetra-1-one and α-caryophyllene, whereas in Bangladesh these components have been reported as triquinacene 1,4-bis (methoxy), (Z)-ocimene and terpinen-4-ol (Bhuiyan et al., 2008; Kamazeri et al., 2012). These differences of chemical composition of the Z. cassumunar essential oil may be caused by variation in growth environment, harvesting period, geographical distribution (Yang et al., 2005).

3.2. Isolation and identification of compounds 1 and 2 from Zingiber cassumunar essential oil

To identify the active constituents of Zingiber cassumunar essential oil that repel mosquitoes, we used silica gel column chromatography to fractionate the essential oil into four fractions (Fr.1, Fr.2, Fr.3 and Fr.4). Our bioassay indicated that Fr.2 had mosquito repellent properties and was therefore further separated by silica gel column chromatography to successively obtain compounds 1 and 2, which are the major components of the Z. cassumunar essential oil. After comparing their NMR data with previous reports, we identified compound 1 as (−)-terpinen-4-ol and compound 2 as (E)-1-(3,4-dimethoxyphenyl)butadiene (DMPBD). The structures of compounds 1 and 2 are shown in Fig.1.

(−)-Terpinen-4-ol (1): colorless oil, [α]D29 +15.88 (c 0.170, acetone); Rf = 0.65 (silica, PE/CHCl3 1:1), Vanillin as chromogenic agent; 1H NMR (500 MHz, CDCl3) δ: 5.30 (1H, dt, J = 4.9, 3.5, 1.7 Hz, H-2), 2.17 (1H, m, H-6a), 2.14 (1H, m, H-3a), 1.92 (2H, m, H-3b, 6b), 1.68 (3H, s, H-10), 1.65 (2H, m, H-7, 5a), 1.55 (1H, ddd, J = 13.4, 11.3, 6.0 Hz, H-5b), 0.94 (3H, d, J = 6.9 Hz, H-8 or 9), 0.92 (3H, d, J = 6.9 Hz, H-8 or 9); 13C-NMR (125 MHz, CDCl3) δ: 134.0 (C-1), 118.6 (C-2), 71.9 (C-4), 36.9 (C-7), 34.7 (C-3), 30.9 (C-5), 27.2 (C-6), 23.4 (C-10), 17.0 (C-8), 17.0 (C-9).

(E)-1-(3,4-Dimethoxyphenyl)butadiene (DMPBD, 2): white solid; Rf = 0.58 (silica, PE/CHCl3 1:1), Vanillin as chromogenic agent; 1H NMR (CDCl3, 500 MHz) δ: 6.96 (1H, d, J = 2.0 Hz, H-2), 6.94 (1H, dd, J = 8.0, 2.0 Hz, H-6b), 6.82 (1H, d, J = 8.0 Hz, H-5b), 6.67...
3.3. Mosquitoes repellent activity

We evaluated the mosquito repellent activity of *Zingiber cassumunar* essential oil and its major constituents on *Aedes albopictus* using an in-cage mosquito repellent model (Table 2). Compared to the reference standard N,N-diethyl-3-methylbenzamide (DEET) (0.03 ± 0.01 mg/cm²). *Z. cassumunar* essential oil showed moderate repellent activity with a MED of 0.16 ± 0.01 mg/cm². To identify the major active components in the *Z. cassumunar* essential oil, we evaluated the mosquito repellent activity of all four fractions of *Z. cassumunar* essential oil. Fr.1, Fr.3, and Fr.4 showed weak mosquito repellent activity. In contrast, Fr.2 showed moderate repellent activity with a MED of 0.25 ± 0.01 mg/cm². As mentioned in the previous section, Fr.2 was subsequently purified to obtain two major components, compounds 1 and 2, which were then tested for their mosquito repellent activity. Compound 1 exhibited moderate mosquito repellent activity with a MED of 0.19 ± 0.0 mg/cm². Compound 2 showed weak repellent activity with a MED of 0.75 ± 0 mg/cm². These results suggest that the major mosquito repellent ingredient in *Z. cassumunar* essential oil is compound 1. The results of repellent bioassay were summarized in Table 2.

Previous research has indicated that the combination of *Zingiber cassumunar* and Sweet basil (Ocimum basilicum) essential oil can be used as both a repellent and a feeding deterrent against mosquitoes (Phukerd and Soonwea, 2014). In the present work, we firstly identified that the active constituent of *Z. cassumunar* essential oil that repels mosquitoes is (−)-terpinen-4-ol (1). This finding was consistent with previous studies that found that (−)-terpinen-4-ol is (1) derived from Cryptomeria japonica repels *Ae. aegypti* and *Ae. albopictus* (Gu et al., 2009).

3.4. Larvicidal activity

We evaluated toxicities of *Zingiber cassumunar* essential oil and its major components against first instar larvae of *Aedes albopictus* by larvicidal bioassay (Table 3). The larvicidal assay indicated that *Z. cassumunar* essential oil is strongly toxic to first instar larvae of *Ae. albopictus*, with a LC₅₀ of 44.86 µg/L after 24 h treatment. Compounds 1 and 2 showed no toxicity under the highest tested dosage (104 mg/mL). We also evaluated the toxicity of Fr. 1, which contained 70% sabine. However, Fr.1 showed no toxicity under the highest tested dosage (104 mg/mL). The blank control group (DMSO) also showed no toxicity. Therefore, larvicidal activity of the *Zingiber cassumunar* essential oil was not due to its three major components. Other minor constituents of the *Z. cassumunar* essential oil were probably responsible for the larvicidal activity.

Previous studies have identified several chemical constituents of *Zingiber cassumunar* essential oil (α-terpine, γ-terpinene, β-myrcone, terpinolene, β-pinene, (+)-limonene, and limonene) that show larvicidal activities against *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* (Cheng et al., 2009a,b; Dias et al., 2014; Govindarajan et al., 2012; Haribalan et al., 2009). In addition, pleurobynthanos isolated from *Z. cassumunar* essential oil also exhibited larvicidal effects against *Kaempferia rotunda* in a chronic feeding bioassay (Nugroho et al., 1996). DMPBD isolated from *Z. cassumunar* has shown larvicidal activity against the second instar of *Ae. aegypti* and ovicidal activity against the bruchid *Callosobruchus maculatus* (Bandara et al., 2005). However, *Z. cassumunar* essential oil obtained from Malaysia shows no activity against the four instar of *Ae. aegypti*, likely because the chemical composition of the rhizome oil from Malaysia differs from those of other countries (Jantan et al., 2003).

3.5. Adulticidal activity

We evaluated the adulticidal activities of the *Zingiber cassumunar* essential oil, compounds 1, 2, and sabine (substituted by Fr.1, which contained more than 70% sabine) against adults of *Aedes albopictus* using a fumigating bioassay. Compound 2 and sabine did not result in mosquito death at the testing concentration of 6.4% after fumigating 24 h. The blank control (acetone) showed no toxicity after fumigating 24 h. Compound 1 showed moderate adulticidal activity, while compound 2 exhibited potent adulticidal activity (Table 4). Essential oil concentrations of 25.6% (v/v) led to 100% mortality of mosquitoes after fumigating 4 h. Compound 1 concentrations of 6.4% led to 100% mortality after fumigating 1 h. After fumigating 24 h, the LC₅₀ of *Z. cassumunar* essential oil and compound 1 were 5.44% and 2.10%, respectively.

The effect of fumigation time of *Zingiber cassumunar* essential oil and compound 1 were evaluated by LT₅₀ (Table 5). The LT₅₀ of *Z. cassumunar* essential oil and compound 1 were 14.33 min and 15.05 min under the concentration of LC₉₀ at 0.5 h. These findings show that compound 1 possesses remarkable adulticidal activity and is one of the major that shows adulticidal activity against *Ae. albopictus*.

Previous studies have reported that *Zingiber cassumunar* essential oil and compound 1 show fumigant and contact toxicities against *Tribolium castaneum* and *Lasioderma serricorne* adults (Wang et al., 2015). Additional main constituents of *Z. cassumunar* essential oil have been suggested to possess fumigant toxicity against *Ae. aegypti*, including 1, 8-cineole, p-cymene, γ-terpinene, 4-terpinolene, and α-pinene (Lucia et al., 2013). We speculate that *Z. cassumunar* essential oil possesses insecticidal and adulticidal activities for mosquitoes and insects, and compound 1 may be the

| Table 2 | Larvicidal activity of *Zingiber cassumunar* essential oil against first instar larvae of *Aedes albopictus*. |
|---------|---------------------------------------------------------------------------------------------------------|
| Regression equation | LC₅₀ (95%CI)μg/L | LC₉₀ (95%CI)μg/L |
| Essential oil | Y = 0.04X-1.58 | 44.86 (36.85–55.94) | 81.33 (67.34–107.30) |
| LC₅₀ and LC₉₀ were the concentration of causing 50% and 90% mortality against larvae after 24 h; 95%CI: 95% confidence intervals. |
Z. cassumunar vary from place to place. These bio-activities of Z. cassumunar plant, some new compounds with bio-activity were also discovered in our previous study of non-volatile chemical components of this conducted visualization, methodology, and investigation. Hong-Xia Zhang conducted methodology and investigation. Hong-Zheng Sun and Hong-Hai Su were in charge of breeding and identification of the major active constituent responsible for its activities. Other minor constituents of Z. cassumunar essential oil, such as α-pinene and γ-terpinene, may also play a synergistic role in adulticidal activity.

4. Conclusion

In this work, we verified that Zingiber cassumunar essential oil acts as a mosquito repellent against Aedes albopictus and identified the main active compound as (−)-terpinen-4-ol. These findings are consistent with the traditional uses of Z. cassumunar essential oil in Dai medicine. This study firstly find that Z. cassumunar essential oil shows larvicidal activity against the first instar of Ae. albopictus. Moreover, we found that Z. cassumunar essential oil and its major components exhibit fumigant toxicity against Ae. albopictus adults, and that (−)-terpinen-4-ol is responsible for this adulticidal activity. These results suggest that Z. cassumunar essential oil possesses potential to be applied in plant-derived repellents and adulticides. (−)-Terpinen-4-ol can also be used in prevention and control of mosquitoes and insects as an important repellent and adulticidal active substance.

The present study found that chemical constituents of essential oil of Zingiber cassumunar from different regions are very different. In our previous study of non-volatile chemical components of this plant, some new compounds with bio-activity were also discovered (Li et al., 2019). That is to say, the secondary metabolites of Z. cassumunar vary from place to place. These bio-activities of Z. cassumunar from Yunnan may be related to the specificity of its chemical compositions. So it is important to protect plants in different habitats.

Author contribution

Ming-Xiang Li conducted experiments, validation, formal analysis, investigation, and wrote the manuscript. Yong-Peng Ma conducted visualization, methodology, and investigation. Hong-Xia Zhang conducted methodology and investigation. Hong-Zheng Sun and Hong-Hai Su were in charge of breeding and identification of the mosquitoes. Sheng-Ji Pei collected and identified the plant material. Zhi-Zhi Du conducted supervision, conceptualization, writing-Review & Editing, project administration, and funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

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