Chemical Compositions of Crassocephalum crepidioides Essential Oils and Larvicidal Activities Against Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus

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Abstract
The leaf, stem, and floral essential oils of Crassocephalum crepidioides growing wild in central Vietnam were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry. The major component in all 3 oils was myrcene (59.3%, 26.1%, and 43.3%, respectively). The 24-hour mosquito larvicidal activities of the oil of the aerial parts (stems and leaves) were determined against wild-caught Aedes albopictus (IC50 = 14.3 μg/mL), laboratory-reared Aedes aegypti (IC50 = 4.95 μg/mL), and wild-caught Culex quinquefasciatus (IC50 = 18.4 μg/mL). The high concentration of myrcene in the essential oil likely accounts for the mosquito larvicidal activity observed.

Keywords
mosquito, vector control, dengue fever, myrcene

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Crassocephalum crepidioides (Benth.) S. Moore (Asteraceae) had been treated as synonymous with Gynura crepidioides Benth., Gynura sarcobensis DC., and Senecio diversifolius A. Rich., but the current accepted name is C. crepidioides.1 The plant is native to tropical Africa but has been introduced throughout Asia,1 including China,2 Japan,3 and Thailand4 as well as Vietnam,5 where it has become an aggressive weed. It is consumed as a nutraceutical vegetable in Benin6 and Nigeria7 as well as Vietnam.8 However, the plant is known to contain the pyrrolizidine alkaloids jacobine and jacoline.9 Medicinally, C. crepidioides is used in Cameroon to get rid of intestinal worms,10 in Uganda to treat human immunodeficiency virus/AIDS,11 and in the Ivory Coast by pregnant women “to make the baby move.”12

Previous reports on the essential oils of C. crepidioides have appeared, including leaf essential oils from Cameroon,13 Nigeria,14 India,15 and China16; floral15 and root essential oils from India17; and stem oil from Nigeria.14

Mosquitoes have been and continue to be the most deadly creatures on earth. Culex quinquefasciatus Say (Diptera: Culicidae), the southern house mosquito, is a vector of lymphatic filariasis18 as well as several arboviruses such as West Nile virus and St Louis encephalitis virus19 and possibly Zika virus.20 The Aedes group of mosquitoes includes the Asian tiger mosquito, Aedes albopictus (Skuse), and the yellow fever...
mosquito, *Aedes aegypti* (L.). Both *Ae. albopictus* and *Ae. aegypti* are known vectors of yellow fever virus, chikungunya virus, dengue virus, and Zika virus. Dengue fever epidemics are frequent and widespread in Vietnam and chikungunya and Zika infections have been reported. In this work, we have investigated the chemical compositions and mosquito larvicidal activities of essential oils of *C. crepidioides* collected from wild-growing plants in central Vietnam.

The chemical compositions of *C. crepidioides* from Vietnam are listed in Table 1. The major components of the floral essential oil were myrcene (43.3%), β-phellandrene (10.7%), and cryptone (8.1%). The leaf essential oil from the other hand, had myrcene (26.1%), α-pinene (10.7%), α-humulene (5.9%), and (E)-β-ocimene (6.4%). The stem oil, on the other hand, had myrcene (59.3%), β-phellandrene (11.9%), and cryptone (8.1%). The leaf essential oil was also rich in myrcene (43.3%), β-phellandrene (26.1%), α-pinene (26.2%), α-humulene (10.3%), β-caryophyllene (10.3%), and farnesene (30.6%) as major components. The root essential oil of *C. crepidioides* from India was rich in α-pinene (26.1%), α-pinene (59.3%), α-humulene (10.7%), and cryptone (8.1%). The leaf essential oil from India had 46.1% myrcene and 31.0% β-phellandrene whereas the aerial parts essential oil had 45.3% myrcene and 20.2% β-phellandrene. One component, cryptone, that was relatively abundant in the essential oils from Vietnam was only observed in small concentrations (0.1%) in the oil from India. The root essential oil of *C. crepidioides* from India had 46.1% myrcene and 31.0% β-phellandrene whereas the aerial parts essential oil had 45.3% myrcene and 20.2% β-phellandrene. One component, cryptone, that was relatively abundant in the essential oils from Vietnam was only observed in small concentrations (0.1%) in the oil from India. The root essential oil of *C. crepidioides* from India was rich in (E)-β-farnesene (30.6%), α-humulene (10.3%), β-caryophyllene (7.2%), cis-β-guaiene (6.1%), and α-bulnesene (5.3%).

The mosquito larvicidal activities of aerial parts (leaves and stems) essential oil of *C. crepidioides* are summarized in Table 2. The essential oil showed excellent larvicidal activity against all 3 mosquito species tested, comparable to many other essential oils screened for mosquito larvicidal activity. The larvicidal activities of the essential oil of *C. crepidioides* are likely due to the high concentration of myrcene. Myrcene has demonstrated larvicidal activity against *Ae. aegypti* (LC₅₀ = 35.8 µg/mL) and *Ae. albopictus* (LC₅₀ = 27.0 µg/mL), as well as *Culex pipiens* (LC₅₀ = 33.8 µg/mL). Interestingly, myrcene was shown to be relatively inactive as a contact toxin to either *Sitophilus oryzae* or *Tribolium castaneum*, but did exhibit good fumigant

| RIa | Compound name | Leaf | Stem | Floral |
|-----|---------------|------|------|--------|
| 932 | α-Pinene      | 1.2  | 10.7 | 1.3    |
| 948 | Camphene      | -    | 0.3  | -      |
| 971 | Sabinene      | 0.4  | 0.8  | 0.3    |
| 976 | β-Pinene      | trb  | 1.0  | 0.3    |
| 987 | Myrcene       | 59.3 | 26.1 | 43.3   |
| 1023 | p-Cymene     | 0.6  | 0.8  | 0.8    |
| 1028 | Limonene      | 2.4  | 1.5  | 2.0    |
| 1029 | β-Phellandrene| 11.9 | 2.7  | 10.7   |
| 1033 | (Z)-β-Ocimene| -    | 0.2  | -      |
| 1044 | (E)-β-Ocimene| 0.8  | trb  | -      |
| 1049 | 2,6-Dimethyl-2,6-octadiene | 1.6 | 0.9  | 1.6    |
| 1097 | Perillene     | 1.6  | 0.9  | 1.4    |
| 1098 | Linalool      | -    | 1.0  | 0.5    |
| 1144 | trans-Verbenol| -    | 0.3  | -      |
| 1179 | Terpinen-4-ol | -    | 0.3  | -      |
| 1185 | Cryptone      | 6.4  | 2.2  | 8.1    |
| 1188 | trans-4-Caranone | -   | -    | 0.4    |
| 1196 | cis-4-Caranone| -    | -    | 0.4    |
| 1241 | Cuminal       | 0.4  | 0.3  | 1.3    |
| 1279 | Neryl formate | 1.2  | 0.8  | 1.5    |
| 1282 | Bornyl acetate| -    | 3.0  | -      |
| 1289 | p-Cymen-7-ol  | 1.1  | 0.5  | 1.8    |
| 1317 | 3-Hydroxycineole | 0.3 | -    | 0.5    |
| 1320 | 4-Hydroxycryptone | 0.4 | -    | 0.8    |
| 1336 | 3-Oxo-p-menth-1-en-7-al | 0.5 | -    | 0.8    |
| 1355 | Neryl acetate | -    | -    | 0.4    |
| 1364 | Unidentifiedf | 0.5  | -    | 0.9    |
| 1374 | α-Copaene     | 0.7  | 3.2  | 3.3    |
| 1387 | β-Elemene     | -    | 2.3  | -      |
| 1401 | Cyperene      | -    | 1.2  | -      |
| 1418 | β-Caryophyllene| 0.8 | 4.4  | 1.1    |
| 1423 | (3E)-4,8-Dimethyl-3,7-nonadien-2-ol | 1.1 | 0.9  | 1.3    |
| 1431 | trans-α-Bergamotene | -   | -    | 0.4    |
| 1440 | Unidentifiedf | 1.3  | 0.7  | 1.9    |
| 1450 | (E)-β-Farnesene| -   | 5.2  | 0.5    |
| 1454 | α-Humulene    | 0.8  | 5.9  | 3.1    |
| 1480 | Unidentifiedf | 0.9  | 0.7  | -      |
| 1486 | Unidentifiedf | 1.4  | -    | 2.0    |
| 1486 | Germacrene D  | -    | 1.9  | -      |
| 1487 | β-Selinene    | -    | 1.7  | -      |
| 1494 | α-Selinene    | -    | 0.3  | -      |
| 1496 | α-Muurolene   | -    | 0.3  | -      |
| 1497 | Neryl isobutanoate | 0.7 | 0.5  | -      |
| 1515 | δ-Cadinene    | -    | 0.4  | 0.4    |
| 1558 | (E)-Nerolidol | -    | 0.3  | -      |
| 1574 | Spathulenol    | -    | 0.4  | -      |

(Continued)
toxicity against *S. oryzae*. The toxic effects of myrcene may be attributed to neurotoxic effects, but apparently not by inhibition of acetylcholinesterase. Although the larvicidal activity of β-phellandrene has not been reported, a β-phellandrene-rich essential oil of *Cinnamomum scortechinii* (17.3% β-phellandrene) has shown larvicidal activity against both *Ae. aegypti* and *Ae. albopictus* with LC₅₀ of 21.5 and 16.7 µg/mL, respectively.

In order to evaluate potential detrimental environmental effects of the essential oil of *C. crepidioides*, the oil was also screened for toxic effects on nontarget aquatic organisms including the water flea (*Daphnia magna*), the nonbiting midge (*Chironomus tentans*), and the zebrafish (*Danio rerio*) (Table 2). Unfortunately, the essential oil of *C. crepidioides* showed notable toxicity to the nontarget organisms as well. Thus, the essential oil of *C. crepidioides* is probably not an ideal candidate for broad application due to nonselective toxicity; it may still prove useful in smaller, more localized applications such as around homes and buildings. The weedy character and abundance of *C. crepidioides* in Vietnam suggests that availability of the essential oil should not be problematic.

### Experimental

#### Plant Material

Plant materials of *C. crepidioides* (leaves, stems, and flowers) were harvested from plants growing in Hoa Vang district, Da Nang city (16°01′00.6″ N, 108°04′25.6″ E), in June 2018. The plants were identified by Dr. Do Ngoc Dai, and a voucher specimen (LTH129) has been deposited in the Pedagogical Institute of Science, Vinh University. Fresh plant materials were kept at room temperature (≈25°C); 2 kg samples of each of the plant materials were shredded and hydrodistilled for 4 hours using a Clevenger-type apparatus.

| Table 1. | Continued |
|---|---|
| RI | Compound name | Leaf | Stem | Floral |
| 1580 | Caryophyllene oxide | - | 5.0 | 1.1 |
| 1596 | cis-Bisabol-11-ol | - | 0.3 | - |
| 1607 | Humulene epoxide II | - | 3.8 | 2.2 |
| 1619 | Cyperotundone A | - | 0.6 | - |
| 1639 | τ-Cadinol | - | 1.4 | - |
| 1641 | τ-Muurolol | - | 0.4 | - |
| 1653 | α-Cadinol | - | 0.9 | 0.4 |
| 1656 | Selin-11-en-4α-ol | - | 0.7 | - |
| 1662 | cis-Calamenen-10-ol | - | 0.3 | - |
| 1837 | Phytone | - | 0.3 | - |
| 2102 | Phytol | 0.7 | 1.1 | 0.5 |
| Monoterpene hydrocarbons | 79.7 | 46.0 | 61.6 |
| Oxygenated monoterpenoids | 11.0 | 8.9 | 16.5 |
| Sesquiterpene hydrocarbons | 2.4 | 26.9 | 8.8 |
| Oxygenated sesquiterpenoids | 0.0 | 13.6 | 4.0 |
| Diterpenoids | 0.7 | 1.4 | 0.5 |
| Others | 1.1 | 0.9 | 1.3 |
| Total identified | 94.8 | 97.6 | 92.8 |

| Table 2. | Mosquito Larvicidal and Nontarget Toxicity, µg/mL (95% Confidence Intervals in Parentheses), of Essential Oil of *Crassocephalum crepidioides*. |
|---|---|
| *Crassocephalum crepidioides* | |
| *Aedes albopictus* (wild) | |
| IC₅₀ (24 h) | 14.33 (13.30-15.50) |
| IC₉₀ (24 h) | 20.86 (18.82-24.25) |
| *Aedes aegypti* (laboratory) | |
| IC₅₀ (24 h) | 4.95 (4.48-5.45) |
| IC₉₀ (24 h) | 10.28 (8.98-12.32) |
| *Culex quinquefasciatus* (wild) | |
| IC₅₀ (24 h) | 18.44 (16.76-20.29) |
| IC₉₀ (24 h) | 34.01 (29.87-40.42) |
| *Daphnia magna* | |
| IC₅₀ (24 h) | 1.85 (1.73-1.99) |
| IC₉₀ (24 h) | 2.54 (2.31-2.91) |
| *Chironomus tentans* | |
| IC₅₀ (24 h) | 7.29 (6.76-7.91) |
| IC₉₀ (24 h) | 10.48 (9.44-12.19) |
| *Danio rerio* | |
| IC₅₀ (24 h) | 16.87 (15.75-18.13) |
| IC₉₀ (24 h) | 22.39 (20.58-25.07) |
| *Permethrin* (positive control) | | |
| *Aedes albopictus* (wild) | |
| IC₅₀ (24 h) | 0.0021 (0.0018-0.0024) |
| IC₉₀ (24 h) | 0.0049 (0.0040-0.0065) |
| *Culex quinquefasciatus* (wild) | |
| IC₅₀ (24 h) | 0.0165 (0.0149-0.0181) |
| IC₉₀ (24 h) | 0.0305 (0.0267-0.0367) |

*RI = Retention index based on a homologous series of normal alkanes on a ZB-5 capillary column.*
*br = trace (<0.05%).*
*Mass spectrometry [MS (EI)]: 140 (12%), 139 (100%), 121 (55%), 91 (25%), 93 (27%), 83 (33%), 81 (40%), 79 (42%), 69 (68%), 67 (22%), 55 (35%), 43 (50%), 41 (35%).*
*MS (EI): 168 (24%), 139 (48%), 125 (78%), 98 (55%), 97 (66%), 81 (35%), 79 (45%), 71 (38%), 70 (49%), 69 (95%), 55 (53%), 43 (70%), 41 (100%).*
*MS (EI): 208 (14%), 121 (18%), 119 (20%), 109 (30%), 95 (18%), 83 (29%), 82 (32%), 79 (27%), 69 (100%), 55 (24%), 41 (81%).*
*MS (EI): 208 (2%), 152 (8%), 139 (20%), 110 (28%), 109 (18%), 100 (20%), 97 (19%), 96 (19%), 82 (66%), 81 (100%), 71 (37%), 69 (31%), 55 (26%), 43 (38%), 41 (40%).*
The essential oil from the aerial parts of *C. crepidioides* was obtained in 1.35% yield.

**Gas Chromatographic Mass Spectral Analysis**

Each of the essential oils of *C. crepidioides* was analyzed by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Ultra operated in the electron impact (EI) mode (electron energy = 70 eV), scan range = 40 to 400 AMU, scan rate = 3.0 scans/s, and GC-MS solution software. The GC column was a ZB-5 fused silica capillary column (30 m length, 0.25 mm internal diameter), with a (5% phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 µm. The carrier gas was helium with a column head pressure of 552 kPa and flow rate of 1.37 mL/min. Injector temperature was 250°C and the ion source temperature was 200°C. The GC oven temperature was programmed for 50°C initial temperature, with temperature increased at a rate of 2°C/min to 260°C. A 5% w/v solution of the sample in CH2Cl2 was prepared and 0.1 µL was injected with a splitting mode (30:1). Identification of the oil components was based on their RIs determined with reference to a homologous series of n-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the literature, and stored in our in-house Sat-Set library.

**Mosquito Larvicidal Assay**

Larvae of *Ae. aegypti* were collected from a mosquito colony maintained at Laboratory of Parasitology and Entomology of Duy Tan University, Da Nang Vietnam. Wild larvae of *Ae. albopictus* and *Cx. quinquefasciatus* were collected from Hoa Khanh Nam district (16°03′14.9″ N, 108°09′31.2″ E). For the assay, aliquots of the aerial parts (leaves and stems) essential oil of *C. crepidioides* dissolved in dimethyl sulfoxide (DMSO, 1% stock solution) were placed in a 500-mL beaker and added to water that contained 20 larvae (third and early fourth instar). With each experiment, a set of controls using DMSO was also run for comparison. Mortality was recorded after 24 hours of exposure during which no nutritional supplement was added. The experiments were carried out at 25°C ± 2°C. Each test was conducted with 4 replicates with 5 concentrations (12, 6, 3, 1.5, and 0.75 µg/mL). The assay against *C. tentans* larvae was carried out similarly with 4 replicates with 5 concentrations (100, 50, 25, 12.5, and 6 µg/mL). For the assay against *D. rerio*, young, immature fish around 2 to 3 cm in size were selected for the experiment. Twenty fish were separated in 2500-mL plastic containers with 1000 mL of tap water, with a temperature of 25°C ± 2°C and external relative humidity of 85%. For each concentration (100, 50, 25, 12.5, and 6 µg/mL), 4 repetitions of the experiment were performed. The mortality of each nontarget organism was determined following an exposure period of 24 hours. With each experiment, a set of controls using DMSO was also run for comparison.

**Declaration of Conflicting Interests**

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