Original Article

Natural Product Communications
June 2019: 1–5

DOI: 10.1177/1934578X19850033
journals.sagepub.com/home/npx

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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* (IC_{50} = 14.3 μg/mL), laboratory-reared *Aedes aegypti* (IC_{50} = 4.95 μg/mL), and wild-caught *Culex quinquefasciatus* (IC_{50} = 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

Received: February 16th, 2019; Accepted: February 22nd, 2019.

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 Thailand[4] as well as Vietnam,[5] where it has become an aggressive weed. It is consumed as a nutraceutical vegetable in Benin[6] and Nigeria[7] 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 China[16]; floral[15] and root essential oils from India[17]; 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 filariasis[18] as well as several arboviruses such as West Nile virus and St Louis encephalitis virus[19] and possibly Zika virus.[20] The *Aedes* group of mosquitoes includes the Asian tiger mosquito, *Aedes albopictus* (Skuse), and the yellow fever

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mosquito, *Aedes aegypti* (L.). Both *Ae. albopictus* and *Ae. aegypti* are known vectors of yellow fever virus, chikungunya virus, dengue virus, and Zika virus.[21] Dengue fever epidemics are frequent and widespread in Vietnam[22] and chikungunya and Zika infections have been reported.[23] 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 was also rich in myrcene (59.3%), β-phellandrene (11.9%), and cryptone (6.4%). The stem oil, on the other hand, had myrcene (26.1%), α-pinene (10.7%), α-humulene (5.9%), and (*E*)-β-farnesene (5.2%) as the major components. The compositions of the essential oils from Vietnam are in marked contrast to previous reports from other geographical locations. The leaf and stem essential oil compositions of *C. crepidioides* from Nigeria have been reported.[14] However, the compositions cannot be considered reliable. Ten of the 35 listed components are not found in the *Dictionary of Natural Products*,[24] several other listed components have retention times that are too far off to be correct, and the sum of the percentages is >100%. In contrast, the leaf essential oil from Guangzhou, China, showed α-pinene (26.2%) and myrcene (27.4%) as major components.[16] However, this compositional analysis is also unreliable; the elution order of many of the compounds is incorrect and the authors did not determine Kovats retention indices (RIs) for verification of identification. The floral and aerial parts essential oils from the Western Ghats of India[15] have compositions comparable to those from Vietnam. The floral 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.[15] 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%).[17]

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.[25,26] 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* (LC_{50} = 35.8 μg/mL) and *Ae. albopictus* (LC_{50} = 27.0 μg/mL),[27] as well as *Culex pipiens* (LC_{50} = 33.8 μg/mL).[28] Interestingly, myrcene was shown to be relatively inactive as a contact toxin to either *Sitophilus oryzae* or *Tribolium castaneum*, but did exhibit good fumigant

Table 1. Chemical Compositions of Essential Oils of *Crassocephalum crepidioides*.

RI[a]	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	tr[b]	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	tr[b]	-
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	Unidentified[c]	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	(3*E*)-4,8-Dimethyl-3,7-nonadien-2-ol	1.1	0.9	1.3
1431	*trans*-α-Bergamotene	-	-	0.4
1440	Unidentified[d]	1.3	0.7	1.9
1450	(*E*)-β-Farnesene	-	5.2	0.5
1454	α-Humulene	0.8	5.9	3.1
1480	Unidentified[e]	0.9	0.7	-
1486	Unidentified[f]	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)

Table 1. Continued

RI[a]	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

[a]RI = Retention index based on a homologous series of normal alkanes on a ZB-5 capillary column.
[b]tr = trace (<0.05%).
[c]Mass spectrometry [MS (EI)]: 140 (12%), 139 (100%), 121 (55%), 91 (25%), 93 (27%), 83 (53%), 81 (40%), 79 (42%), 69 (68%), 67 (22%), 55 (35%), 43 (50%), 41 (35%).
[d]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%).
[e]MS (EI): 208 (14%), 121 (18%), 119 (20%), 109 (30%), 95 (18%), 83 (29%), 82 (32%), 79 (27%), 69 (100%), 55 (24%), 41 (81%).
[f]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%).

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)	
IC_{50} (24 h)	14.33 (13.30-15.50)
IC_{90} (24 h)	20.86 (18.82-24.25)
Aedes aegypti (laboratory)	
IC_{50} (24 h)	4.95 (4.48-5.45)
IC_{90} (24 h)	10.28 (8.98-12.32)
Culex quinquefasciatus (wild)	
IC_{50} (24 h)	18.44 (16.76-20.29)
IC_{90} (24 h)	34.01 (29.87-40.42)
Daphnia magna	
IC_{50} (24 h)	1.85 (1.73-1.99)
IC_{90} (24 h)	2.54 (2.31-2.91)
Chironomus tentans	
IC_{50} (24 h)	7.29 (6.76-7.91)
IC_{90} (24 h)	10.48 (9.44-12.19)
Danio rerio	
IC_{50} (24 h)	16.87 (15.75-18.13)
IC_{90} (24 h)	22.39 (20.58-25.07)
	Permethrin (positive control)
Aedes albopictus (wild)	
IC_{50} (24 h)	0.0021 (0.0018-0.0024)
IC_{90} (24 h)	0.0049 (0.0040-0.0065)
Culex quinquefasciatus (wild)	
IC_{50} (24 h)	0.0165 (0.0149-0.0181)
IC_{90} (24 h)	0.0305 (0.0267-0.0367)

toxicity against *S. oryzae*.[29] The toxic effects of myrcene may be attributed to neurotoxic effects,[30] but apparently not by inhibition of acetylcholinesterase.[31] 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 LC_{50} of 21.5 and 16.7 μg/mL, respectively.[32]

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.

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 CH_2Cl_2 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,[33] and stored in our in-house Sat-Set library.[34]

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 (negative control) and permethrin (positive control) were also run for comparison. Mortality was recorded after 24 hours of exposure during which no nutritional supplement was added. The experiments were carried out 25°C ± 2°C. Each test was conducted with 4 replicates with several concentrations (100, 50, 25, 12.5, 6, and 3 µg/mL). The acute larvicidal effects on *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* were recorded 24 hours after treatment. Permethrin was used as a positive control. The data obtained were subjected to log-probit analysis[35] to obtain LC_{50} values, LC_{90} values, and 95% confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

Nontarget Lethality Assays

For the assay against *D. magna*, aliquots of essential oil (stems and leaves) of *C. crepidioides*, dissolved in DMSO (1% stock solution), were placed in 250-mL beakers and added to water that contained 20 larvae (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.

Acknowledgments

P.S. and W.N.S. participated in this work as part of the activities of the Aromatic Plant Research Center (APRC, https://aromaticplant.org/).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by Duy Tan University.

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