Phytotoxic and Fungitoxic Activities of the Essential Oil of Kenaf (*Hibiscus cannabinus* L.) Leaves and Its Composition

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The chemical composition of the essential oil of kenaf (*Hibiscus cannabinus*) was examined by GC-MS. Fifty-eight components were characterized from *H. cannabinus* with (*E*)-phytol (28.16%), (*Z*)-phytol (8.02%), *n*-nonanal (5.70%), benzene acetaldehyde (4.39%), (*E*)-2-hexenal (3.10%), and 5-methylfurfural (3.00%) as the major constituents. The oil was phytotoxic to lettuce and bentgrass and had antifungal activity against *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, and *Colletotrichum accutatum* but exhibited little or no algicidal activity.

Keywords: *Kenaf; Hibiscus cannabinus; essential oil composition; fungi; algae; phytotoxicity; allelopathy*

INTRODUCTION

Biochemical interactions between plants (allelopathy) and between plants and other organisms influence plant growth and development in biotic communities. Scientists, worldwide, have recognized these allelochemical activities because they offer potential beneficial uses in agriculture, including decreasing reliance on synthetic herbicides (*1*, *2*), insecticides (*3*), and nematicides (*4*).

Kenaf, *Hibiscus cannabinus* L., is a member of the Malvaceae family that is related to cotton (*Gossypium hirsutum* L.) and okra [*Abelmoschus esculentus* (L.) Moench]. This annual plant native to Africa is being considered as an alternative agricultural crop in many parts of the United States. Kenaf fiber has been evaluated for use in paper, pulp, twines, burlap, animal bedding, ropes, and fishing nets (*5*). Kenaf mulch in vegetable production reduced weed populations and provided initial evidence that kenaf might have allelopathic properties (*6*). Kenaf extracts reduced germination of redroot pigweed (*Amaranthus retroflexus* L.) by 50−70% and to a lesser extent that of Italian ryegrass (*Lolium multiflorum* Lam.) and tomato (*Lycopersicon esculentum* Mill.) (*7*). Extracts from kenaf leaves also decreased germination of redroot pigweed, tomato, Italian ryegrass, and cucumber (*Cucumis sativus* L.) (*8*).

To our knowledge, the only previous report on the leaf volatile constituents of *H. cannabinus* concerns a biotype collected in Egypt (*9*). The authors mention the presence of 10 components, that is, ethyl alcohol, isobutyl alcohol, limonene, phellandrene, α-terpenyl acetate, citral, and four unidentified components. Our work is the first report on the composition of the essential oil from leaves of *H. cannabinus*. In addition, phytotoxic, algicidal, and antifungal activities of the essential oil and its major components were investigated in an effort to discover novel, potentially environmental friendly natural products of use to agriculture.

EXPERIMENTAL PROCEDURES

Plant Material. *H. cannabinus* cv. Everglades 41 was grown at the South Central Research Laboratory in Lane, OK. Fresh leaf samples (harvested on October 31, 1999, 186 days after planting) were collected by removing all of the leaves from each plant. A composite sample was made and stored in a labeled plastic bag at −20 °C until steam-distilled. A voucher specimen was deposited at the South Central Research Laboratory in Lane, OK.

Plant Extraction. *Organic Solvent Extraction.* Kenaf leaves (2 × 1 kg) in MeOH (2 × 2.5 L) were ground in a blender for 2 min. The resulting fine mixture was extracted with MeOH (3 × 5 L) at room temperature for 10 days to give 105 g (yield = 5.2%) of combined crude extract. The MeOH extract (100 g) was suspended in MeOH (400 mL), diluted with water (400 mL). The solution was extracted three times subsequently with hexane, ether, CH_2Cl_2, and EtOAc to give 3.1, 2.7, 2.1, and 11.2 g of dried fractions respectively, after complete drying under reduced pressure.

Water Extract. Extraction of air-dried kenaf leaves was carried out as described by Russo et al. (*7*), using water as a solvent.

All extracts, that is, MeOH, hexane, ether, methylene chloride, ethyl acetate, and water, were tested for phytotyoxic, fungicidal, and algicidal activities.

Essential Oil Isolation and Chemical Characterization. Steam distillation and analyses were conducted as previously described (*10*, *11*) on 26 g of plant material. The oil of *H. cannabinus* was analyzed by GC-MS on a Varian Chrompack Cp-3800 GC coupled with a Varian Chrompack Saturn 2000 GC-MS/MS, equipped with a DB-5 column (30 m × 0.25 mm fused silica capillary column, film thickness = 0.25 μm); injector temperature, 220 °C; transfer line temperature, 240 °C; column temperature, 60−240 °C at 3 °C/min; carrier gas, He; amount injected, 1 μL (split ratio 1:20); ionization energy, 70 eV. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds or by comparison of their retention

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indices and mass spectra with those in the literature (*11*). The relative amounts (RA) of individual components of the oil are expressed as percent peak area relative to total peak area. Clear yellow oil was obtained in a yield of 28 mg (0.11% of fresh weight).

Essential Oil Major Components. The standards (*E*)-phytol, (*Z*)-phytol, *n*-nonanal, benzene acetaldehyde, (*E*)-2-hexenal, and 5-methylfurfural were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Phytotoxicity Assays. Bioassays for the phytotoxic activity of the *H. cannabinus* extracts, essential oil, and major components of the essential oil were carried out as previously reported for lettuce (*Lactuca sativa* cv. Iceberg) and bentgrass (*Agrostis stolonifera* cv. Pencross) in 24-well plates (*12*). *n*-Pentane was used as the transfer solvent for the essential oil and each major component of the essential oil. The loading solvent, *n*-pentane, was allowed to evaporate completely at room temperature (5 min) before dilution with water. An added control with *n*-pentane (evaporated at room temperature) was used to account for possible solvent effects. The effect of the compounds on the growth of the seedlings was observed after 7 days. The phytotoxicity was rated on a scale ranging from 0 to 5. Ratings of 0 and 5 meant no effect and complete inhibition, respectively. Factors used in the evaluation include inhibition of root or shoot growth, as well as overall appearance (i.e., presence of chlorotic and/or necrotic areas on the leaves). Each experiment included duplicate treatments, and the experiments were repeated three times.

Algicidal Assays. The possible toxic effect of the *H. cannabinus* extracts, essential oil, and major components of the essential oil on representative species of algae and cyanobacteria (blue-green algae) was determined according to the general procedure described by Schrader et al. (*13*) using isolates of the cyanobacterium *Oscillatoria perornata* Skuja and the green alga *Selenastrum capricornutum* Printz. Continuous culture systems were used to provide a source of algal and cyanobacterial cells growing at a steady rate.

The essential oil or its major components were dissolved in *n*-pentane and placed into 96-well quartz microplates (Hellma Cells, Inc., Forest Hills, NY). Quartz microplates were required, because *n*-pentane was used as the loading solvent and is not compatible with polystyrene microplates (*14*). After the *n*-pentane in the essential oil and each of the major component dilutions was evaporated from the microplate wells, either cyanobacterial or unialgal culture was added (200 μL). Three replications were used for each dilution (undiluted, 1:10, 1:100, and 1:1000) and each control, and the experiment was repeated. Plates were placed in a growth chamber maintained at 28−30 °C and illuminated by overhead fluorescent lights (20 W, cool white) at a photon flux density of 21−27 μeinstein/m^2/s. Optical densities of each well were measured at 650 nm in 24-h intervals for 4 days using a Packard model Spectracount microplate reader (Packard Instrument Co., Meriden, CT). Mean values of the optical density (cell biomass) measurements for each dilution and control were graphed to help determine toxic selectivity of the diluted *H. cannabinus* oil.

Fungicidal Assays. Pathogen production and inoculum preparation for *Colletotrichum fragariae* Brooks, *Colletotrichum gloeosporioides* Penz. & Sacc., and *Colletotrichum accutatum* Simmonds were carried out in the usual manner as described in detail elsewhere (*15*). Conidia concentrations were determined photometrically (*15*, *16*) from a standard curve, and suspensions were then adjusted with sterile distilled water to a concentration of 1.0×10^6 conidia/mL. Bioautographic assays (*17*) were chosen to determine the inhibition of fungal growth on thin-layer chromatography (TLC) plates. Hexane, ether, methylene chloride, and ethyl acetate extracts were dissolved in pesticide grade acetone. The MeOH extract was dissolved in HPLC grade MeOH. The water extract was dissolved in distilled water. *n*-Pentane was used as the transfer solvent for the essential oil or each major component of the essential oil. *H. cannabinus* extracts, essential oil, and major components of the essential oil were individually spotted on a TLC plate using a disposable glass micropipet to load 400.0, 200.0, 100.0, 50.0, 25.0, 10.0, and 1.0 μg of each component.

Table 1. Constituents of the Essential Oil of the Leaves of *H. cannabinus*[a]

component	RT	% RA	RI
n-octane	2.61	1.39	800
furfural	3.00	1.98	832
(*E*)-2-hexenal	3.29	3.10	853
2,4-(*E*,*E*)-hexadienal	4.32	0.14	909
2-acetylfuran	4.39	0.18	912
5-methylfurfural	5.55	3.00	963
trans-*p*-menthane	6.00	0.06	978
3-octanone	6.15	0.10	986
6-methyl-5-hepten-2-one	6.24	0.17	986
3-*p*-menthene	6.28	1.13	987
m-mentha-1(7),8-diene	6.71	1.48	1000
benzene acetaldehyde	8.00	4.39	1043
acetophenone	8.80	0.10	1065
m-tolualdehyde	8.87	0.04	1067
n-octanol	9.03	0.26	1070
cis-linalool oxide	9.12	0.31	1074
p-cymenene	9.73	0.17	1089
2-nonanol	10.14	0.21	1098
n-nonanal	10.30	5.70	1102
4-ketoisophorone	11.79	0.12	1143
β-pinene oxide	12.21	0.82	1154
trans-pinocamphone	12.50	0.48	1160
p-mentha-1,5-dien-8-ol	12.82	0.11	1167
cis-pinocamphone	13.03	0.45	1173
p-methylacetophenone	13.47	0.12	1182
α-terpineol	13.83	0.15	1190
myrtenol	13.95	0.41	1196
n-decanal	14.49	0.28	1204
trans-carveol	15.08	0.68	1218
(*E*)-2-decenal	16.89	0.56	1262
perilla aldehyde	17.43	0.65	1271
α-terpinen-7-al	17.79	0.09	1281
indole	18.11	0.28	1288
(*E*,*Z*)-2,4-decadienal	18.29	0.08	1290
(*E*,*E*)-2,4-decadienal	19.25	0.60	1314
(*E*)-β-damascenone	22.25	0.33	1382
geranylacetone	25.19	0.39	1453
allo-aromadendrene	25.55	0.17	1461
ethyl (*E*)-cinnamate	25.61	0.14	1463
β-chamigrene	26.17	0.31	1475
β-(*E*)-ionone	26.55	1.61	1484
β-cadinene	28.12	2.14	1523
α-calacorene	28.89	0.73	1542
globulol	30.64	0.33	1584
cis-β-elemenone	30.98	0.31	1592
khusimone	31.15	0.67	1595
γ-eudesmol	32.42	0.25	1630
α-bisabobol oxide B	33.82	0.44	1654
cadalene	34.07	0.75	1674
benzyl benzoate	37.30	0.20	1761
n-nonadecane	40.39	1.40	1900
methyl hexadecanoate	43.19	0.14	1925
ethyl hexadecanoate	45.49	1.14	1994
(*Z*)-phytol	48.33	8.02	2083
(*E*)-phytol	49.26	28.16	2113
n-tricosane	54.88	0.36	2300
n-tetracosane	57.70	0.27	2400
n-pentacosane	60.43	1.16	2500

[a] Abbreviations: RI, retention index as determined on a DB-5 column using the homologous series of *n*-hydrocarbons; RT, retention time on a DB-5 column in minutes; RA, relative area (peak area relative to total peak area).

The loading solvents, *n*-pentane, MeOH, acetone, and water, were used as controls. Each experiment was repeated three times. To detect biological activity directly on the TLC plate, the silica gel plates (250 μm, Silica Gel GF Uniplate, Analtech, Inc., Newark DE) were sprayed with a spore suspension as previously described (*18*). Positive antifungal activities (Table 3) were established by the presence of measurable zones of growth inhibition. The antifungal activity was recorded as the diameter of the clear zone (in millimeters) after 4 days of incubation for each fungus (Table 3).

Table 2. Phytotoxic Activity of Kenaf Essential Oil

	visual rating[a]	
treatment, mg/mL	lettuce	bentgrass
0	0	0
0.01	0	0
0.03	0	0
0.1	3	3
0.3	4	4
1	5	5

[a] Visual rating scale ranges from 0 to 5 for no effect to 100% inhibition.

RESULTS AND DISCUSSION

In our laboratory, biological evaluation of kenaf leaf extracts showed no phytotoxic, fungicidal, or algicidal activity. However, Russo et al. (*7*) showed that kenaf had allelopathic activities and that weathering of its tissues led to decreasing allelopathic activities. One possibility is that active volatile compounds may be lost during the weathering process. Therefore, the composition and activity of the essential oil was investigated.

A light yellow essential oil of *H. cannabinus* was obtained in a yield of 0.11% fresh weight. Results of the GC-MS analysis of the oil are shown in Table 1, where the components are listed in order of their elution from the DB-5 column. Fifty-seven constituents accounting for >79% of the total oil composition were identified. None of the unidentified compounds accounted for >1.00% of the total area. Four monoterpene hydrocarbons (2.84%) and five sesquiterpene hydrocarbons (2.84%) were identified in the oil. Among oxygenated compounds, alcohols (39.34%) were most predominant in the oil, followed by carbonyl compounds (30.79%). Diterpenes (36.18%) constituted most of the alcohol compounds; the main components were (*E*)-phytol (28.16%) and (*Z*)-phytol (8.02%). Aldehydes (20.32%) constituted most of the carbonyl compounds; the main components were *n*-nonanal (5.70%), benzene acetaldehyde (4.39%), (*E*)-2-hexenal (3.10%), and 5-methylfurfural (3.00%).

The oil of *H. cannabinus* was phytotoxic to lettuce and bentgrass seeds, with 80−100% growth reduction observed at 0.3 mg/mL (Table 2). The oil was also moderately active on both species at 0.1 mg/mL, but did not completely inhibit the growth of the seedlings completely at this concentration. No effect was observed at lower concentrations. The above findings may explain the loss of allelopathic effect on seed germination as the length of the time of weathering of kenaf increases (*7*). Weathering might lead to the loss of the volatile allelopathic components, which might reasonably be expected to be present in much higher concentrations in the oil than in the extracts. All major components were tested for phytotoxic activity under the same condition as the essential oil [*n*-pentane (loading solvent) was allowed to completely evaporate]. No phytotoxic activity was observed with the highly volatile (*E*)-2-hexenal and *n*-nonanal, both reported to be phytotoxic (*19*), even at a treatment level as high as 2 mg/mL. These results may be due to the very low boiling point of both compounds (i.e., highly volatile). No phytotoxic activity was observed for any of the remaining major components of the kenaf oil.

The oil was tested against one cyanobacterium and one green alga for potential as a selective cyanobactericide. The oil was only slightly active against *O. perornata* at the highest concentration (250 μg/mL), and no activity was observed for the green algae (data not shown). No algicidal activity was observed for the oil major components when tested under the same conditions as the essential oil.

Filamentous fungi of the genus *Colletotrichum* and its teleomorph *Glomerella* are major plant pathogens worldwide. *Colletotrichum* species often cause typical symptoms of anthracnose, a disease characterized by sunken necrotic lesions usually bounded by a red margin (*22*, *23*). The pathogens *Colletotrichum acutatum* J. H. Simmonds, *C. gloeosporioides* Penz., and *C. fragariae* A. N. Brooks can occur singly or in combination and can infect flowers, fruit, leaves, petioles, stolons, and crowns (*24*, *25*). New approaches to anthracnose disease control are necessary as the effectiveness and availability of commercial fungicides decrease (*26*, *27*). The oil of *H. cannabinus* showed minimal inhibitory activity to the growth of plant pathogenic fungi (Table 3). Zones of inhibition were determined using TLC bioautography for *C. fragariae*, *C. gloeosporioides*, and *C. accutatum* at 400 and 100 μg of oil. No inhibition was determined at 50 μg. When the major components of the oil were tested for antifungal activity, only 5-methylfurfural, *n*-nonanal, and benzene

Table 3. Fungal Growth Inhibition of the Essential Oil of *H. cannabinus* and Its Major Active Components on *C. gloeosporioides*, *C. fragaria*, and *C. accutatum*

		diameter of zone of inhibition in mm[a]			
organism tested	amount, μg	essential oil	5-methylfurfural	benzene acetaldehyde	*n*-nonanal
C. gloeosporioides	400	10.0 (±1.5)	13.0 (±1.0)	9.0 (±1.4)	30.0[b]
	200	7.0 (±1.0)	10.0 (±0.5)	7.0 (±1.0)	30.0[b]
	100	5.0 (±1.0)	8.0 (±1.0)	5.0 (±1.0)	17.0 (±1.2)
	50	0.0 (0.0)	6.0 (±1.2)	0.0 (0.0)	9.0 (±1.4)
	25	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C. fragariae	400	12.0 (± 1.2)	10.0 (±1.0)	10.0 (±1.0)	30.0[b]
	200	10.0 (±1.0)	8.0 (± 0.8)	8.0 (±0.5)	30.0[b]
	100	6.0 (± 0.5)	5.0 (±1.0)	6.0 (±1.2)	18.0 (±1.5)
	50	0.0 (0.0)	4.0 (±0.5)	0.0 (0.0)	10.0 (±1.0)
	25	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
C. accutatum	400	10.0 (± 1.4)	13.0 (±1.0)	10.0 (±1.0)	30.0[b]
	200	8.0 (± 1.0)	9.0 (±1.0)	7.0 (±1.4)	30.0[b]
	100	6.0 (± 0.8)	7.0 (±1.0)	5.0 (±12)	16.0 (±1.2)
	50	0.0 (0.0)	5.0 (±1.2)	0.0 (0.0)	10.0 (±1.5)
	25	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a] Average of clear zones of inhibition. Standard deviation is give in parentheses. [b] Complete test area was inhibited.

acetaldehyde showed weak antifungal activities, with zones of inhibition for 100 μg of 5-methylfurfural, 100 μg of *n*-nonanal, and 50 μg of benzene acetaldehyde (see Table 3).

In conclusion, the phytotoxic activity observed with 0.1 mg/mL oil suggests that some of the oil's components may be active. The demonstrated phytotoxic activity of the oil may explain the loss of allelopathic effect on seed germination by weathering or biodegradation of the kenaf leaves (*7*). The observed phytotoxic activity of the essential oil is probably due to the minor, less volatile component(s). In this study, kenaf oil and its components do not provide any strong leads for use as selective algicides or fungicides. We are currently undertaking the process of bioassay-guided isolation and identification of the phytotoxic active compound(s) in the oil.

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