PERGAMON

Phytochemistry 61 (2002) 37–40

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thunb.

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Received 15 January 2002; received in revised form 30 May 2002

Abstract

The essential oil from the leaves of *Callicarpa japonica* was analyzed by GC–MS, and 84 compounds were identified. The main constituents of the essential oil were spathulenol (18.1%), germacrene B (13.0%), bicyclogermacrene (11.0%), globulol (3.3%), viridiflorol (2.6%), α-guaiene (2.3%), and γ-elemene (2.0%). The essential oil constituents of *C. japonica* were significantly different from those found in our previous work on *Callicarpa americana*. The oil of *C. japonica* was selectively phytotoxic to bentgrass compared to lettuce seeds, with 80–100% growth reduction observed at 0.3 mg/ml. Published by Elsevier Science Ltd.

Keywords: *Callicarpa japonica*; Verbenaceae; Essential oil composition; Bioactivity; Phytotoxicity

1. Introduction

Callicarpa japonica Thunb. (Verbenaceae), Japanese beautyberry is a multi-stemmed, deciduous shrub reaching a height of up to 1.2–2.0 m, native to Japan, and hardy to zone 5. Past studies on this species led to the isolation of 5,6,7-trimethoxyflavone (Hosozawa et al., 1972). This compound was reported to possess biological activities, such as piscicidal (Hosozawa et al., 1972) and antiviral properties (Tsuchiya et al., 1985; Toshimitsu et al., 1997). We have reported that the essential oil of *Callicarpa americana* L. was selectively toxic toward the cyanobacterium *Oscillatoria perornata,* responsible for musty off-flavor problems in catfish (Tellez et al., 2000). Our research now focuses on the essential oil of *C. japonica*. To our knowledge, this is the first study on the composition and phytotoxic activity of this *Callicarpa* species.

2. Results and discussion

A light yellow essential oil of *C. japonica* was obtained in a yield of 0.1% 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. Eighty-four constituents, accounting for more than 79% of the total oil composition, were identified. Twenty-two sesquiterpene hydrocarbons (35%), 21 oxygenated monoterpenes (3.14%), 18 oxygenated sesquiterpenes (32%), and 5 oxygenated diterpenes (2%) were identified in the oil. The main components of the essential oil from *C. japonica* were spathulenol (18.1%), germacrene B (13.0%), bicyclogermacrene (11.0%), globulol (3.3%), viridiflorol (2.6%), α-guaiene (2.3%), and γ-elemene (2.0%). Only one unknown in *C. japonica* accounted for $>0.5\%$ of the total area (RA). This compound (KI 980) accounted for 4.6% RA and shows m/z (relative intensity) 210 $[M^+]$ (12), 95 (23), 81 (32), and 69 (100).

The essential oil constituents of *C. japonica* were significantly different from our previous work on the related species indigenous to North America, *C. americana* (Table 1). A total of 137 components were found within both *C. americana* and *C. japonica* essential oils. Sixteen of those components were identical, leaving 121 different components between the two species. Of the 16 components found in both species, none were predominant in *C. japonica*.

The oil of *C. japonica* was phytotoxic to bentgrass (*Agrostis stolonifera* cv. Pencross, monocotyledon) seedlings, with 80–100% growth reduction observed at 0.3 mg/ml. The oil was also moderately active on bentgrass at 0.1 mg/ml, not completely inhibiting the growth

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0031-9422/02/$ - see front matter Published by Elsevier Science Ltd.
PII: S0031-9422(02)00207-8

Table 1
Constituents of the oil of *C. japonica* and *C. americana*

Components	KI[a]	% RA[b] *C. japonica*	% RA *C. americana*
Hexanal	800	–[c]	0.5
Furfural	831	t	–
(*E*)-2-Hexenal	853	1.3	4.6
(*Z*)-3-Hexenol	858	–	0.8
2-Hexen-1-ol	868	–	0.2
n-Hexanol	866	t[d]	0.3
2,4-(*E*-*E*)-Hexadienal	906	t	–
Cumene	927	t	–
α-Thujene	931	t	0.1
α-Pinene	938	0.1	2.5
Camphene	952	t	t
Benzaldehyde	960	t	t
Unknown	980	4.6	–
1-Octen-3-ol	980	–	8.5
β-Pinene	981	–	8.8
3-Octanone	987	–	0.1
Mesitylene	994	0.1	–
3-Octanol	995	–	t
(*E*)-3-Hexanol acetate	1005	0.1	–
(*Z*)-3-Hexenol acetate	1006	–	t
(*E*,*E*)-2,4-Heptadienal	1010	–	t
α-Terpinene	1019	–	t
p-Cymene	1027	–	0.6
Limonene	1032	–	0.4
Benzyl alcohol	1033	–	0.2
1,8-Cineole	1034	–	0.1
Benzene acetaldehyde	1043	0.2	0.7
γ-Terpinene	1062	–	0.1
trans-arbusculone	1072	–	t
cis-Linalool oxide	1074	–	t
Terpinolene	1090	–	t
Trans-sabinene hydrate	1098	0.1	–
Linalool	1099	–	0.3
cis-Thujone	1101	t	–
n-Nonanal	1103	–	t
Phenyl ethyl alcohol	1108	t	–
Endo-fenchol	1114	–	t
Dehydro-sabina ketone	1117	t	–
cis-*p*-Menth-2-en-1-ol	1122	–	t
α-Campholenal	1127	–	t
Nopinone	1137		0.1
Trans-pinocarveol	1138	t	–
trans-Sabinol	1140	–	0.2
4-Keto-isophorone	1141	t	t
Myrcenone	1145	t	–
Pinocarvone	1163	–	0.2
Borneol	1167	–	t
p-Mentha-1,5-dien-8-ol	1168	–	t
Terpin-4-ol	1176	t	0.3
α-Terpineol	1188	t	0.2
Methyl salicylate	1190	0.1	–
Myrtenal	1193	t	0.2
Myrtenol	1194	t	t
n-Dodecane	1198	0.1	–
Trans-dihydro carvone	1202	0.1	–
Trans-carveol	1218	1	–
Isobornyl formate	1228	t	–
Geraniol	1254	t	–
(*E*)-2-Decenal	1260	t	–
Isobornyl acetate	1285	t	–
Indole	1287	t	–
Carvacrol	1298	t	–
Sesamol	1312	0.4	–
δ-Elemene	1337	1.0	–
Eugenol	1355	1.0	–
(*Z*)-β-Damascenone	1359	t	–
Cyclosativene	1367	0.1	–
Isoledene	1372	0.1	–
α-Ylangene	1372	–	t
α-Copaene	1375	t	0.2
β-Patchoulene	1378	t	–
(*E*)-β-Damascenone	1381	0.4	t
β-Bourbonene	1385	–	0.3
β-Elemene	1390	1.0	–
α-Gurjunene	1407	0.3	–
E-Caryophyllene	1416	t	0.6
β-Gurjunene	1429	–	t
γ-Elemene	1433	2.0	–
α-Guaiene	1442	2.3	–
Khusimene	1447	0.2	–
α-Humulene	1454	–	10.1
Seychellene	1459	1.0	–
γ-Muurolene	1477	–	0.7
Germacrene D	1479	1.0	–
Unknown sesquiterpene $C_{15}H_{24}$	1483	–	1.1
β-Selinene	1485	0.4	2.2
cis-β-Guaiene	1489	0.2	–
Valencene	1492	–	3.5
α-Selinene	1494	–	0.5
Bicyclogermacrene	1495	11.0	–
α-Muurolene	1499	–	0.2
Germacrene A	1501	0.2	–
trans-β-Guaiene	1503	0.2	–
γ-Cadinene	1511	0.2	0.3
7-*epi*-α-Selinene	1516	–	1.3
δ-Cadinene	1521	0.3	0.5
cis-Calamene	1522	–	t
Cadina-1,4-diene	1532	t	–
α-Cadinene	1537	–	t
Selina-3,7(11)-diene	1539	0.5	–
α-Calacorene	1542	–	t
Elemol	1549	0.5	–
Germacrene B	1556	13.0	–
Ledol	1565	1.3	–
Spathulenol	1577	18.1	–
Caryophyllene oxide	1581	–	1
Globulol	1582	3.3	–
Viridiflorol	1589	2.6	–
Unknown sesquiterpene $C_{15}H_{24}O$	1596	–	1.6
Humulene epoxide II	1606	–	13.9
Unknown sesquiterpene $C_{15}H_{24}O$	1614	–	1.2
Unknown sesquiterpene $C_{15}H_{24}O$	1626	–	2.0
Unknown sesquiterpene $C_{15}H_{24}O$	1630	–	5.8
epi-α-Cadinol	1638	0.4	0.6
Cubenol	1641	–	0.6
α-Muurolol	1645	–	0.5
Selin-11-en-4-α-ol	1651	1.1	–
α-Cadinol	1653	–	2.2

(*continued on next page*)

Table 1 (*continued*)

Components	KI[a]	% RA[b] *C. japonica*	% RA *C. americana*
7-*epi*-α-Eudesmol	1657	–	9.5
Khusinol	1677	–	0.3
(*Z*)-α-Santalol	1679	0.4	–
8-Cedren-13-ol	1687	0.2	–
Juniper camphor	1691	1.3	–
Curcuphenol	1714	1.7	–
6*R*,7*R*-Bisabolone	1737	0.1	–
β-Acoradienol	1757	0.2	–
(*Z*)-Lanceol	1762	0.5	–
Unknown, *m*/*z* 324 [M^+], 175 (100)	1804	–	4.3
α-Vetivone	1836	0.1	–
Isohibaene	1926	0.1	–
epi-13-Manool	1958	0.1	–
Dolabradiene	1970	t	–
Occidol acetate	1974	0.1	–
epi-13-Manoyl oxide	2008	0.5	–
Abietatriene	2051	0.2	–
(*E*)-Phytol	2111	0.2	–
Isopimarol	2305	1.0	–

[a] KI: Kovats' indices as determined on a DB-5 column using the homologous series of *n*-hydrocarbons.
[b] RA: relative area (peak area relative to total peak area).
[c] –: Not found.
[d] t: trace (<0.1%).

of seedlings at this concentration. No effect was observed at lower concentrations. No phytotoxic activity of the essential oil of *C. japonica* was observed towards lettuce (*Lactuca sativa* cv. Iceberg, dicotyledon) at 1.0 mg/ml. These results suggest selective toxicity of *C. japonica* oil towards monocotyledons species. The oil was also tested for antifungal, termiticidal, and cyanobacterial activity but no promising activities were found (data not shown).

The phytotoxic activity against grass, observed with 0.1 mg/ml of *C. japonica* essential oil, is not surprising, since some of the oil components that account for 1% or greater of the oil, such as eugenol (Gant and Clebsch, 1975; Bessette, 2000), ledol and viridiflorol (Terrom, 1994), are known to be phytotoxic.

3. Experimental

3.1. Plant materials

Leaves of *C. japonica* were collected in late August from three plants growing in Lafayette County, Mississippi. At latitude 34° 20′ north and longitude 89° 40′ west, ~15 km east of Oxford, MS. A voucher specimen of this plant was deposited at the National Center for Natural Products Research (Voucher No. 63823). The plants growing in Mississippi were grown from cuttings of *C. japonica* taken from the arboretum of James A. Duke (Ethnobotanist, The Herbal Village, 8210 Murphy Road, Fulton, Maryland 20759). Dr. Jucile McCook, curator of the University of Mississippi Herbarium confirmed the identification of the species.

3.2. Essential oil isolation and chemical characterization

Steam distillation and analyses were conducted as previously described (Tellez et al., 1999; Adams, 1995) on 30 g of plant material, yielding 28 mg (0.1% of fresh weight) clear yellow oil. The oil of *C. japonica* was analyzed by GC–MS on a Varian Chrompack Cp-3800 GC coupled with 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 retention indices and mass spectra with those in the literature (Adams, 1995, 2001). The relative amounts (RA) of individual components of the oil are expressed as percent peak area relative to total peak area.

3.3. Phytotoxicity assays

Bioassays for phytotoxic activity of the *C. japonica* essential oil was carried out as previously reported for lettuce (*Lactuca sativa* cv. Iceberg) and bentgrass (*Agrostis stolonifera* cv. Pencross) in 24-well plates (Dayan et al., 2000). *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 adding water to the wells. An added control with *n*-pentane, evaporated at room temperature, was used to account for possible solvent effects. The effect of *C. japonica* essential oil 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.

Acknowledgements

We thank Dr. James A. Duke for the gift of the original plant.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing, Carol Stream, IL.

Adams, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectroscopy. Allured Publishing, Carol Stream, IL.

Bessette, S.M., 2000. Essential Oils and their Components as Herbicides. Ecosmart Technologies, Inc., USA, 24 pp., US 99-123174 19990305, Application: WO 2000-2000US5478 20000303.

Dayan, F.E., Romagni, J.G., Duke, S.O., 2000. Investigating the mode of action of natural phytotoxins. J. Chem. Ecol. 26, 2079–2094.

Gant, R.E., Clebsch, E.E.C., 1975. Allelopathic influences of *Sassafras alibidum* in old-field succession in Tennessee. Grad. Program Ecol. 56, 604–615.

Hosozawa, S., Kato, N., Munakata, K., 1972. 5,6,7-Trimethoxyflavone from *Callicarpa japonica*. Phytochemistry 11, 2362.

Tellez, M.R., Canel, C., Rimando, A.M., Duke, S.O., 1999. Differential accumulation of isoprenoids in glanded and glandless *Artemisia annua*. Phytochemistry 52, 1035–1040.

Tellez, M.R., Dayan, F.E., Schrader, K.K., Wedge, D.E., Duke, S.O., 2000. Composition and some biological activities of the essential oil of *Callicarpa Americana* L. J. Agric. Food Chem. 48, 3008–3012.

Terrom, G., 1994. Biocidal and/or Biostatic Compositions and their Applications. Transbiotech, Fr., 19 pp., FR 2697133, Application: FR 92–13182 19921028.

Toshimitsu, O., Hidaeki, O., Takeda, Y., 1997. Antiviral activity of 5,6,7-trimethoxyflavone and its potentiation of the antiherpes activity of acyclovir. J. Antimicrob. Chemother. 39, 821–824.

Tsuchiya, Y., Shimizu, M., Hiyama, Y., Itoh, K., Hashimoto, Y., Nakayama, M., Horie, T., Morita, N., 1985. Antiviral activity of natural occurring flavonoids *in vitro*. Chem. Pharm. Bull. 33, 3881–3886.