Fumigant Antifungal Activity of Essential Oil Components from *Acorus gramineus* against Three Phytopathogenic Fungi

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Analysis by gas chromatography-mass spectrometry led to the identification of 26 compounds in *Acorus gramineus* essential oil. The antifungal activity of the identified compounds was tested singularly by using standard compounds. Allyl isothiocyanate and *cis*-asarone showed inhibition rates of 100% against *P. cactorum* at 28 mg/l air. In a test with *C. parasitica* and *F. circinatum*, allyl isothiocyante and *cis*-asarone showed moderate activity at 28 mg/l air.

Key words: Medicinal Plant Essential Oils, Antifungal Activity, *Acorus gramineus*

Introduction

The rhizome of *Acorus gramineus* Solander, belonging to the family Araceae, has been reported to have sedative, digestive, analgesic, diuretic and insecticidal effects (Korean Pharmacognosy Association, 2000; Park *et al*., 2003). Little work has been done to consider its potential to manage forest diseases, although the antifungal activity of extracts of *Arcous* rhizome against *Cladosporium cucumerinum*, *Colletotrichum orbiculare*, *Magnaporthe grisea*, and *Pythium ultimum* has been noted (Lee *et al*., 2004). In this study, we assessed the fungicidal activity of components derived from *A. gramineus* essential oil against three phytopathogenic fungi to find new types of disease control alternatives.

Materials and Methods

Fungal strain and culture conditions

The phytopathogenic fungi used in this study were *Phytophthora cactorum, Cryponectria parasitica* and *Fusarium circinatum.* They were isolated from the Japanese angelica tree (*Aralia elata*), chestnut (*Castanea crenata* var. *dulcis*) and pitch pine (*Pinus rigida*), respectively. These plant pathogens were routinely maintained on potato dextrose agar (PDA) at 28 °C.

Fumigant antifungal activity

Fumigant antifungal activity was examined by the method of Alvarez-Castellanos *et al.* (2001). PDA plates were prepared using plastic Petri dishes (87 mm i.d.). Each agar-mycelial plug (7 mm i.d.) of *P. cactorum* and *C. parasitica* was inoculated at the centre of the dish. The Petri dishes were placed with the lid upside down. Essential oil was introduced onto a paper disc (8 mm i.d.; Advantec, Tokyo, Japan), and the disc was placed on the lid without agar. Petri dishes were sealed with parafilm to prevent the leakage of test oils and compounds. After inoculation, the plates were incubated at 28 °C in the dark. The colony growth diameter was measured after the fungal growth in the control treatment had completely covered the Petri dishes (average 6 d for *P. cactorum*, 8 d for *C. parasitica* and 7 d for *F. circinatum*). All treatments were replicated four times.

Growth inhibition of treatment against control was calculated by percentage, using the formula:

$$\% \text{ inhibition} = (C - T/C) \times 100,$$

where C is the average of 4 replicates of hyphal extension (mm) in the control and T is the average of 4 replicates of hyphal extension (mm) of plates treated with either the essential oil or an individual compound.

Statistical analysis

Antifungal activity was transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by the SAS program (SAS Institute, 1999).

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Results

The chemical constituents and antifungal activity of compounds identified in *A. gramineus* essential oil are shown in Tables I and II. The most abundant compound in *A. gramineus* was *cis*-asarone followed by methyl eugenol, estragole, camphene, and α-pinene. Composition rates of other identified compounds were less than $< 2.0\%$. The antifungal activity varied according to compound and dose. Allyl isothiocyanate showed 100% of inhibition against *P. cactorum* at 28 and 14 mg/l air. The antifungal activity of allyl isothiocyanate reduced to 84.4 and 72.5% at 7 and 3.5 mg/l air, respectively. The inhibition rate of *cis*-asarone was 100% at 28 mg/l air, but decreased to 86.5, 63.7, and 39.9% at 14, 7, and 3.5 mg/l air, respectively. The other compounds did not show antifungal activity against *P. cactorum* at 28 mg/l air. In a test with *C. parasitica*, *cis*-asarone was the most active followed by allyl isothiocyanate and heptanal at 28 mg/l air. Allyl isothiocyanate and *cis*-asarone showed moderate antifungal activity against *F. circinatum* at 28 mg/l air. The other compounds did not show antifungal activity.

Discussion

Fujita *et al.* (1970) reported chemical compositions of essential oils obtained from *A. gramineus* collected from various localities in Japan. The essential oil consisted mainly of *cis*-asarone (63.2–81.2%) and asarone (8.8–13.7%) with small amounts of 32 compounds. The chemical composition was similar to our study, but there was a difference in the ratio of each compound. Ogihara and Takeda (1990) already reported that the essential oil content can vary markedly, depending on growing conditions.

Structure-activity relationships of certain plant compounds against fungi have been well studied. Lee *et al.* (2008) investigated antifungal activities of compounds identified in Myrtaceae essential oils, and reported that primary alcohols were more

Table I. Chemical composition of *Acorus gramineus* essential oil.

Compound	RI[a] on DB-1		RI[a] on FFAP		Area (%)
	Standard	Natural	Standard	Natural	
Hexanal	792	792	1075	nd[b]	0.11
Allyl isothiocyanate	851	851	1354	nd	0.17
Heptanal	879	879	1177	nd	tr[c]
α-Pinene	928	928	1014	1012	2.66
Camphene	940	940	1054	1053	8.06
Sabinene	963	962	1147[d]	nd	0.92
β-Pinene	967	967	1098	1097	1.54
Octanal	979	979	1280	1280	tr
Myrcene	981	981	1155	1153	0.20
p-Cymene	1010	1010	1261	1258	1.10
1,8-Cineole	1018	1018	1196	1193	1.97
Limonene	1020	1020	1189	1186	0.95
cis-Ocimene	1027	1027	1227	1225	1.17
Linalool	1084	1084	1536	1532	1.22
Camphor	1117	1117	1500	1500	1.73
Borneol	1147	1147	1686	1683	0.71
Terpine-4-ol	1159	1159	1590	nd	0.31
α-Terpineol	1170	1170	1685	nd	0.20
Estragole	1173	1173	1661	1658	8.13
Decanal	1183	1183	1488	nd	tr
Citronellol	1211	1211	1753	nd	0.19
Methyl eugenol	1370	1370	2005	2005	9.30
β-Caryophyllene	1414	1414	1583	1585	0.40
Unknown	–	1421	–	2082	5.86
α-Humulene	1447	1447	1656	nd	0.11
Unknown	–	1537	–	2233	9.00
cis-Asarone	1582	1582	2309	2306	22.10
trans-Asarone	1635	1635	2409	2404	0.46
Sum					78.57

[a] van Den Dool and Kratz retention (1963) index on DB-1 and FFAP columns, according to *n*-alkanes (C_9–C_{16}, DB-1; C_7–C_{20}, FFAP). Components were identified by co-injection with authentic standard on two columns.
[b] Not detected.
[c] Trace (< 0.05).
[d] Chung *et al.* (1993).

Table II. Fumigant antifungal activity of constituents from *Acorus gramineus* essential oil against three plant pathogens.

Compound[a]	Concentration (mg/l air)	Inhibition rate (%)		
		P. cactorum	*C. parasitica*	*F. circinatum*
Hexanal	28	0f[b]	0f	0d
Allyl isothiocyanate	28	100a	59.9 ± 4.4ab	63.2 ± 2.0a
	14	100a	47.4 ± 0.7cd	38.6 ± 1.5c
	7	84.4 ± 1.8b	0f	0d
	3.5	72.5 ± 1.7c	–	–
Heptanal	28	0f	52.7 ± 8.0bc	0d
	14	–[c]	0f	–
Octanal	28	0f	0f	0d
cis-Ocimene	28	0f	0f	0d
Decanal	28	0f	0f	0d
cis-Asarone	28	100a	65.0 ± 1.7a	54.3 ± 1.3b
	14	86.5 ± 0.7b	52.1 ± 4.1ab	0d
	7	63.7 ± 1.5d	41.0 ± 1.2d	–
	3.5	39.9 ± 1.6e	28.8 ± 1.3e	–
trans-Asarone	28	0f	0f	0d

[a] Antifungal activities of α-pinene, β-pinene, myrcene, *p*-cymene, 1,8-cineole, limonene, linalool, terpine-4-ol, α-terpineol, citronellol, β-caryophyllene and α-humulene against *P. cactorum, C. parasitica* and *F. circinatum* were tested in a previous study (Lee *et al*., 2008). Antifungal activities of camphor, borneol, estragole and methyl eugenol against *P. cactorum* and *C. parasitica* were tested in another study (Kim *et al*., 2008).
[b] Means within a column followed by the same letters are not significantly different (P = 0.05, Scheffe's test, SAS Institute).
[c] Not tested.

active than secondary or tertiary alcohols. Concerning aldehydes, α,β-unsaturated carbonyl compounds were more toxic. Moleyar and Narasimham (1986) reported that unsaturated aldehydes (citral and cinnamaldehyde) and unsaturated alcohols such as geraniol were more effective against *Aspergillus niger, Fusarium oxysporum* and *Penicillium digitatum* than hydrocarbons such as camphene, limonene and α-terpinene. In our study, allyl isothiocyanate and *cis*-asarone showed antifungal activity against three phytopathogenic fungi. The antifungal activity of *A. gramineus* essential oil was mainly contributed to the action of *cis*-asarone and methyl eugenol, because the ratios of allyl isothiocyanate and borneol were less than 1% in *A. gramineus* essential oil. There was a significant difference in the antifungal activity between *cis*- and *trans*-asarone. The antifungal activity of *cis*-asarone was much more pronounced than of *trans*-asarone. These results indicate that the toxicity of asarones might be due to the *cis*-configuration rather than to the position of the double bond. Furthermore, the same result was also observed studying the insecticidal activity (Park *et al*., 2003).

Our results indicate that *A. gramineus* essential oil and its components could be useful as control agents for phytopathogenic fungi. However, for the practical application of this oil and its single components as novel fungicides, further studies are necessary on the safety of these materials to humans and on the development of formulations to improve the efficacy and stability and to reduce cost.

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