Plant Pathol. J. 22(1) : 94-96 (2006)

The Plant Pathology Journal

Note

In vivo Antifungal Activity Against Various Plant Pathogenic Fungi of Curcuminoids Isolated from the Rhizomes of *Curcuma longa*

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(Received on September 21, 2005; Accepted on December 7, 2005)

In a search for plant extracts with potent *in vivo* antifungal activity against various plant pathogenic fungi, the methanol extract of the *Curcuma longa* rhizomes effectively controlled the development of rice blast caused by *Magnaporthe grisea* and tomato late blight caused by *Phytophthora infestans*. Three curcuminoids such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin were purified from the methanol extract of *C. longa* rhizomes as antifungal principles. Among the three curcuminoids, demethoxycurcumin was the most active to both rice blast and tomato late blight, followed in order by curcumin and bisdemethoxycurcumin. However, they all exhibited no or little *in vivo* antifungal activity against other fungal pathogens causing rice sheath blight (*Corticium sasaki*), tomato gray mold (*Botrytis cinerea*), wheat leaf rust (*Puccinia recondita*), or barley powdery mildew (*Blumeria graminis* f. sp. *hordei*).

Keywords : antifungal activity, *Curcuma longa,* curcuminoids, *Magnaporthe grisea*, *Phytophthora infestans*

Curcuma longa L. (turmeric) belongs to the family Zingiberaceae and its rhizomes have been used as a yellow dye for foods and spices (curry). It has been also used as a natural medicine for the treatment of menstrual disorders, rheumatism, and traumatic diseases in East Asia (Tang and Erenbrand, 1992). Turmeric extract is an oleoresin consisting of a volatile oil (light) fraction and a yellowish-brown color (heavy) fraction. It contains a number of curcuminoids, monoterpenoids, and sesquiterpenoids (Tang and Erenbrand, 1992). The compounds showing yellow color are three curcuminoid compounds, curcumin (1,7-bis(4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione), demethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids have been demonstrated not only to have anticancer activity (Araujo and Leon, 2001; Chun et al., 1999), antileishmanial activity (Gomes et al., 2002), antiviral activity (Araujo and Leon, 2001), and insecticidal (Chander et al., 1991; Chander et al., 1992), repellent (Jilani and Saxna, 1990; Su et al., 1982), and antifeeding activities (Jilani and Saxna, 1990), but also to have antifungal activity (Kim et al., 2003).

Kim et al. (2003) reported that curcumin was isolated from *C. longa* rhizomes as a major antifungal principle and it showed potent *in vivo* antifungal activity against several fungal pathogens causing rice sheath blight (*Corticium sasaki*), tomato late blight (*Phytophthora infestans*), or wheat leaf rust (*Puccinia recondita*). In a search for antifungal compounds from natural medicinal plants for the control of plant diseases, we isolated three curcuminoids showing *in vivo* antifungal activity from *C. longa* rhizomes and then identified them as curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Cho et al., 2005). During the study, however, we found that curcumin showed different antifungal spectrum against plant diseases from the result previously reported by Kim et al. (2003), and demethoxycurcumin has higher *in vivo* antifungal activity than curcumin. This paper reports the characterization of curcuminoids and their *in vivo* antifungal activity against six plant pathogenic fungi.

Dried and finely powdered rhizomes (800 g) of *C. longa* were purchased from Fine Korea Co. (Seoul, Korea). The powder was extracted twice with methanol (6 L) at room temperature for 1 day and then filtered. The two filtrates were combined and then concentrated to dryness *in vacuo* at 35°C using a rotary evaporator to yield ca. 61 g. The extract was redissolved in 3 L of 80% methanol and then partitioned with equal volumes of *n*-hexane. Both layers were concentrated to dryness. The aqueous layer was redissolved in 3 L of distilled water and then successively extracted twice with equal volumes of ethyl acetate and n-butanol. The organic and aqueous layers were concentrated to dryness under reduced pressure. The four fractions were bioassayed for *in vivo* antifungal activity against six plant diseases. The ethyl acetate extract (35 g) was the most

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active, followed by the butanol extract (ca 3.0 g; Table 1). Three antifungal substances were purified from the ethyl acetate fraction by repeated silica gel and Sephadex LH-20 column chromatography and then identified as curcumin, demethoxycurcumin, and bisdemethoxycurcumin by mass and ^{1}H-NMR spectral data (Cho et al., 2005).

The fractions obtained during the purification of active substances from the rhizomes of *C. longa* and the purified metabolites were tested for *in vivo* antifungal activity against six plant pathogenic fungi such as *M. grisea* (causing rice blast), *C. sasaki* (rice sheath blight), *Botrytis cinerea* (tomato gray mold), *P. infestans* (tomato late blight), *P. recondita* (wheat leaf rust) and *Blumeria graminis* f. sp. *hordei* (barley powdery mildew). The *in vivo* antifungal bioassays were performed as previously described (Kim et al., 2001; Kim et al., 2004). Blasticidin-S (1 and 50 ug/ml) for rice blast, validamycin (5 and 50 μg/ml) for rice sheath blight, fludioxonil (5 and 50 μg/ml) for tomato gray mold, dimethomorph (2 and 10 μg/ml) for tomato late blight, flusilazole (2 and 10 μg/ml) for wheat leaf rust, and benomyl (1 and 100 μg/ml) for barley powdery mildew were applied as positive controls.

As shown in Fig. 1, the methanol extract of *C. longa* rhizomes suppressed the disease development of *M. grisea* on rice plants and *P. infestans* on tomato plants, and moderate *in vivo* antifungal activity against *P. recondita* on wheat plants. However, it was actually inactive to *C. sasaki*, *B. cinerea*, and *B. graminis* f. sp. *hordei*. Further solvent fractionation showed the strongest antifungal activity against *M. grisea*, *B. cinerea*, and *P. recondita* in the ethyl acetate fraction (Table 1), but little antifungal activity was shown in the other fractions, such as the *n*-hexane, butanol and aqueous fractions.

The three curcuminoids were effective in suppressing blast on rice plants and Phytophthora late blight on tomato

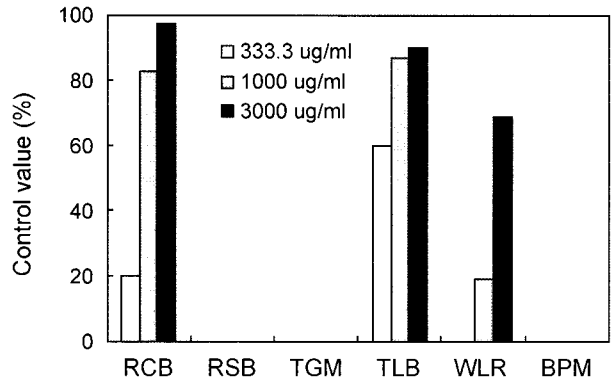

Fig. 1. *In vivo* antifungal activity of a methanol extract of the *Curcuma longa* rhizomes against six phytopathogenic fungi (RCB, rice blast; RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew).

Table 1. *In vivo* antifungal activities of the four fractions obtained by solvent partitioning of a methanol extract of the *Curcuma longa* rhizomes against six plant pathogenic fungi[a]

Fraction	Conc. (μg/ml)	Control value (%)					
		RCB[b]	RSB	TGM	TLB	WLR	BPM
n-Hexane layer	2,000	0[c]	0	6	0	0	0
Ethyl acetate layer	2,000	93	0	19	94	67	0
n-Butanol layer	2,000	52	0	31	0	7	0
Aqueous layer	2,000	13	0	0	29	27	8

[a]Seedlings were inoculated with spores or mycelial suspensions of the test organisms 1 day after spraying with the chemical solutions.
[b]RCB, rice blast (*Magnaporthe grisea*); RSB, rice sheath blight (*Corticium sasaki*); TGM, tomato gray mold (*Botrytis cinerea*); TLB, tomato late blight (*Phytophthora infestans*); WLR, wheat leaf rust (*Puccinia recondita*); BPM, barley powdery mildew (*Blumeria graminis* f. sp. *hordei*).
[c]Numbers are averages of three replications.

Table 2. *In vivo* antifungal activity of three curcuminoids isolated from the *Curcuma longa* rhizomes against six phytopathogenic fungi[a]

Fraction	Conc. (μg/ml)	Control value (%)					
		RCB[b]	RSB	TGM	TLB	WLR	BPM
Curcumin	500	69[c]	0	0	91	0	0
	250	56	0	0	88	0	0
	125	38	0	0	7	0	0
Demethoxy-curcumin	500	89	5	0	96	0	0
	250	85	0	0	94	0	0
	125	13	0	0	64	0	0
Bisdemethoxy-curcumin	500	66	0	0	21	0	0
	250	56	0	0	7	0	0
	125	14	0	0	0	0	0
Blasticidin-S	50	100	–[d]	–	–	–	–
	1	58	–	–	–	–	–
Validamycin	50	–	100	–	–	–	–
	5	–	60	–	–	–	–
Fludioxonil	50	–	–	100	–	–	–
	5	–	–	71	–	–	–
Dimethomorph	10	–	–	–	78	–	–
	2	–	–	–	25	–	–
Flusilazole	10	–	–	–	–	100	–
	2	–	–	–	–	73	–
Benomyl	100	–	–	–	–	–	100
	1	–	–	–	–	–	67

[a]Seedlings were inoculated with spores or mycelial suspensions of the test organisms 1 day after spraying with the chemical solutions.
[b]RCB, rice blast (*Magnaporthe grisea*); RSB, rice sheath blight (*Corticium sasaki*); TGM, tomato gray mold (*Botrytis cinerea*); TLB, tomato late blight (*Phytophthora infestans*); WLR, wheat leaf rust (*Puccinia recondita*); BPM, barley powdery mildew (*Blumeria graminis* f. sp. *hordei*).
[c]Numbers are averages of three replications.
[d]Not tested

plants among the 6 plant diseases tested (Table 2). They displayed the similar antifungal spectra each other. Out of the three curcuminoids, demethoxycurcumin showed the highest *in vivo* antifungal activity, followed in order by curcumin and bisdemethoxycurcumin. Compared to the results reported previously by Kim et al. (2003), the antifungal activities of curcumin against tomato late blight and barley powdery mildew in both studies were similar one another, whereas antifungal activities against other plant diseases such as rice blast, rice sheath blight and wheat leaf rust were quite different. This study revealed that curcumin had antifungal activity against only in rice blast among the three plant diseases. In contrast, Kim et al. (2003) reported that curcumin was not active to rice blast, but rice sheath blight and wheat leaf rust. To elucidate the difference in both studies, curcumin compounds isolated by both research groups should be tested together for *in vivo* antifungal activities against the plant diseases.

On the other hand, Apisariyakul et al. (1995) reported that curcumin has no antifungal activity against dermatophytes, molds, and yeasts *in vitro*, but turmeric oil inhibits dermatophytes and molds *in vitro*. We also found that the curcuminoids isolated from *C. longa* had no or little inhibitory activity against mycelial growth of various plant pathogenic fungi such as *M. grisea*, *B. cinerea*, *C. sasaki*, *Pythium ultimum*, *Alternaria alternata*, *Fusarium oxysporum* and *P. infestans* even at a concentration of 100 μg/ml except *Colletotrichum* species. They all effectively inhibited the mycelial growth of three red pepper anthracnose pathogens, *C. coccodes*, *C. gloeosporioides*, and *C. acutatum*, in a range of 0.4-100 μg/ml in a dose-dependent manner (Cho et al., 2005). It is very interesting that they effectively suppress rice blast caused by *M. grisea* and tomato late blight caused by *P. infestans*, even though they do not inhibit mycelial growth of the fungi. Mode of action of curcuminoids *in vivo* against *M. grisea* and *P. infestans* should be investigated in future.

Curcuminoids and crude extract of *C. longa* rhizomes showed no phytotoxicity against rice, tomato, wheat, and barley plants even at 2,000 μg/ml and 5,000 μg/ml, respectively. The rhizomes of *C. longa* have been used as a common household medicine and as a spice in Asia including Korea, which indicates no toxicity to humans. We are in progress to develop a natural fungicide using a crude extract of *C. longa* for the control of several plant diseases such as rice blast caused by *M. grisea* and tomato late blight caused by *P. infestans*.

Acknowledgements

This research was supported by a grant (PF0321206-00) from the Plant Diversity Research Center 21st Century Frontier Program funded by the Ministry of Science and Technology of the Korean government.

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