Anthraquinones Isolated from *Cassia tora* (Leguminosae) Seed Show an Antifungal Property against Phytopathogenic Fungi

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The fungicidal activities of *Cassia tora* extracts and their active principles were determined against *Botrytis cineria*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia grisea*, and *Rhizoctonia solani* using a whole plant method in vivo and were compared with synthetic fungicides and three commercially available anthraquinones. The responses varied with the plant pathogen tested. At 1 g/L, the chloroform fraction of *C. tora* showed a strong fungicidal activity against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. Emodin, physcion, and rhein were isolated from the chloroform fraction using chromatographic techniques and showed strong and moderate fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. Furthermore, aloe-emodin showed strong and moderate fungicidal activities against *B. cinerea* and *R. solani*, respectively, but did not inhibit the growth of *E. graminis*, *P. infestans*, *P. recondita*, and *Py. grisea*. Little or no activity was observed for anthraquinone and anthraquinone-2-carboxylic acid when tested at 1 g/L. Chlorothalonil and dichlofluanid as synthetic fungicides were active against *P. infestans* and *B. cinerea* at 0.05 g/L, respectively. Our results demonstrate the fungicidal actions of emodin, physcion, and rhein from *C. tora*.

KEYWORDS: *Botrytis cineria*; *Cassia tora*; emodin; *Erysiphe graminis*; fungicidal activity; physcion; phytopathogenic fungi; *Phytophthora infestans*; *Puccinia recondita*; *Pyricularia grisea*; rhein; *Rhizoctonia solani*

INTRODUCTION

Plant diseases are estimated to cause yield reductions of almost 20% in the major food and cash crops worldwide (*1–4*). Synthetic fungicides have effectively controlled plant diseases for a number of years, but increasing concern over environmental effects of the currently used fungicides has highlighted the need for the development of alternative types of selective control or for methods of crop protection with the reduced use of conventional fungicides (*2, 3*). Research into plant natural products is now being intensified as it becomes evident that plant-derived fungicides still have an enormous potential to influence modern agrochemical research (*3*). Although it is difficult to define the ecological significance of most of the synthetic fungicides, there is good reason to suppose that the secondary metabolism of plants has evolved to protect them from attack by microbial pathogens (*3*).

Plant extracts or their constituents may provide an alternative to currently used synthetic fungicides to control phytopathogenic fungi, because they constitute a rich source of bioactive chemicals (*4, 5*). Because these are often active against a limited number of specific target species, are biodegradable to nontoxic products, and are potentially suitable for use in integrated management programs, they could lead to the development of new classes of possibly safer disease control agents. Therefore, efforts have focused on secondary plant metabolites for potentially useful products as commercial fungicides or as lead compounds (*6–8*). The current authors already reported and confirmed that among 25 seed extracts of leguminous species, the extract of *Cassia tora* seeds exhibits potent fungicidal activities against *Pyricularia grisea*, *Botrytis cinerea*, *Phytophthora infestans*, and *Erysiphe graminis* (*9*). *C. tora* is not only important as a source of natural fungicides but is also considered important for its medicinal properties, such as an antiseptic, antidiarrheal, antioxidant, and antimutagen (*10–12*). However, relatively little work has been done on the management of fungi or their damage using the *Cassia* species despite their excellent biological actions (*9–12*). In the studies described herein, we assessed in vivo the fungicidal activities of *C. tora* seed-derived components, the synthetic fungicides chlorothalonil and dichlofluanid, and three commercially available anthraquinones against six phytopathogenic fungi.

MATERIALS AND METHODS

Chemicals. Anthraquinone, anthraquinone-2-carboxylic acid, and 1,2,4-trihydroxyanthraquinone were purchased from Sigma Chemical (St. Louis, MO), and aloe-emodin was supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing,

Table 1. Test Condition of Phytopathogenic Fungi in a Greenhouse

disease	plant/ stage	plant no./pot	pathogen	inoculation; inoculum dosage	holding period in humidity chamber	chamber temp[a]/ period[b]
RCB	rice/2-leaf	3	*Py. grisea*	leaf spray; 1×10^6 spore/mL	1 day	25 °C/5
RSB	rice/3-leaf	3	*R. solani*	pouring inoculum[c] on the soil; 10 mL/pot	7 days	28 °C/7
CGM	cucumber/1-leaf	1	*B. cinerea*	leaf spray; 1×10^6 spore/mL	3 days	20 °C/3
TLB	tomato/2-leaf	2	*P. infestans*	leaf spray; 1×10^5 zoosporangia/mL	4 days	18 °C/4
WLR	wheat/1-leaf	4	*P. recondita*	leaf spray; 1.5 mg of uredospores/pot	1 day	20 °C/10
BPM	barley/1-leaf	4	*E. graminis*	dusting[d] the conidia on barley plant	no need	20 °C/10

[a] The chamber temperature was kept for treated and control plants. [b] Period (days) from inoculation of pathogen to evaluation of disease severity on host plants; includes the days that plants were needed in the humidity chamber. [c] Inoculum of *R. solani* was made by inoculating mycelial plugs in wheat bran medium at 25 °C for 7 days and macerated at the ratio of 500 g of medium incubated *R. solani* per 1 L of distilled water into the mixer. [d] The reparation of *E. graminis* conidia, known as an obligate parasite, was made by dusting the inoculation of conidia on 10 barley plants cultivated in the pot (ϕ 7.5 cm). The treated plants were dusted with *E. graminis* conidia formed on leaves of barley by the ratio of eight tested pots/maintained pot.

Peoples Republic of China). Chlorothalonil ($C_8Cl_4N_2$, MW 265.90) and dichlofluanid ($C_9H_{11}Cl_2FN_2O_2S_2$, MW 333.24) were purchased from Dongbu HanNong Chemical Co., and all other chemicals were of reagent grade.

Extraction and Isolation. The seeds of *C. tora* were purchased from a local market in Chonju and identified by Prof. Sang-Hyun Lee (Forestry Department, Chonbuk National University, South Korea). *C. tora* seeds (6 kg) were ground in a blender, extracted twice with methanol (12 L) at room temperature for 2 days, and filtered. The combined filtrate was then concentrated in vacuo in a vacuum at 45 °C to yield 11.4% (684 g). Next, the combined filtrate (20 g) was partitioned into hexane (2.8 g), chloroform (4.6 g), ethyl acetate (2.4 g), butanol (2.5 g), and water soluble (7.7 g) portions. This step was repeated 34 times with the organic solvent. The organic solvent portions were concentrated to dryness by rotary evaporation at 45 °C, while the water portion was freeze-dried. Finally, hexane (96 g), chloroform (157 g), ethyl acetate (82 g), butanol (85 g), and water soluble (262 g) portions were obtained.

Because of its good fungicidal activity against *B. cinerea*, *E. graminis*, *P. infestans*, and *Rhizoctonia solani*, the chloroform (15 g) portion was chromatographed on a 95 cm × 6.5 cm (i.d.) silica gel column (Merck 70−230 mesh, 900 g) and successively eluted with a stepwise gradient of chloroform/methanol (0, 10, 20, 30, 40, and 50%). The active 30% fraction (7.1 g) showed strong fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. This fraction was further chromatographed on a silica gel column and eluted with chloroform/methanol (30:1). The column fractions were analyzed by thin-later chromatography (TLC) (silica gel 60 F_{254}, chloroform/methanol, 30:1), and fractions with similar TLC patterns were combined. One fraction (4.2 g) showed strong fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*. This bioactive fraction was then chromatographed over a Sephadex LH-20 column (Pharmacia, 800 mm × 49 mm) using chloroform/acetone/methanol (50:1:2). This operation was repeated three times. Again, an active fraction (2.4 g) exhibited strong fungicidal activities against the four test species. This fraction was chromatographed over a Polyclar AT column (Touzart and Matignon, 100 g) packed with chloroform/acetone (50:1, v/v) and eluted with an increasing ratio of methanol (1, 2, 5, 10, and 20%). The active fraction 2 (310 mg, compound I) showed strong fungicidal activities against *B. cinerea*, *P. infestans*, and *R. solani*. Active fraction 4, containing fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*, was finally purified successively on a Sephadex LH-20 column (Pharmacia) eluted with chloroform/methanol (6:4, v/v) and cellulose (Merck) eluted with chloroform/methanol (6:4, v/v). Finally, compounds II (203 mg) and III (215 mg) were isolated. Structural determination of the active isolates (compounds I−III) was made by a spectral analysis. ^1H NMR and ^{13}C NMR spectra were recorded using a Bruker AM-500 spectrometer. The UV spectra were obtained using a Waters 490 spectrometer, IR spectra using a Biorad FT-80 spectrophotometer, and mass spectra using a JEOL JMS-DX 30 spectrometer.

In Vivo Fungicidal Activity. Six plant diseases evaluated in this study were rice blast (RCB), rice sheath blight (RSB), cucumber gray mold (CGM), tomato late blight (TLB), wheat leaf rust (WLR), and barley powdery mildew (BPM) caused by *Py. grisea*, *R. solani*, *B. cinerea*, *P. infestans*, *Puccinia recondita*, and *E. graminis*, respectively (**Table 1**). Except for *P. recondita* and *E. graminis*, the others were routinely maintained on potato dextrose agar (PDA) slants and V-8 agar slants and kept for stock at 4 °C.

The fungicidal activities of test samples were determined by a whole plant method in a greenhouse, as previously described (*7*). The initial concentration of the test solution was 2 g/L, at which over 60% control value of the compounds (aloe-emodin, emodin, physcion, and rhein) showed fungicidal activity against all test fungi; further tests employed a dilution sequence of 1, 0.5, 0.25, 0.125, and 0.0625 g/L.

To prepare test solutions at the concentration of 2 g/L, 100 mg of the test sample was dissolved in 0.5 mL of dimethyl sulfoxide and then diluted with 49.5 mL of water containing Tween 20 (250 µg/mL). Fifty milliliters of each test sample solution was sprayed onto two pots on a turntable at the same time. The treated plants were kept in a greenhouse for 1 day, before being inoculated by each pathogen. The controls were sprayed with the Tween 20 solution. All tests were replicated three times.

In a test with RCB caused by *Py. grisea*, rice plants at the second leaf stage (three plants/pot) were sprayed with each test solution. The treated plants were inoculated with a suspension of conidia in distilled water (1×10^6 spores/mL) and kept in a chamber (25 °C) for 24 h under 100% relative humidity (RH). The treated and control plants were then held in a lighted chamber (26 ± 2 °C; 85% RH; photoperiod, 12 h; light intensity, 3000 lux) for 5 days and rated for disease severity. The severity was assessed on a 0−5 scale where 0 = no disease, 1 = disease affecting <1% area of second leaf, 2 = 1−10%, 3 = 10−25%, 4 = 25−60%, and 5 = >60% of leaf area affected. For RSB caused by *R. solani*, each test solution was sprayed onto rice plants at the third leaf stage (three plants/pot). The plants were inoculated by injecting the inoculum at the base of the plants. The inoculum of *R. solani* was made by inoculating mycelial plugs in wheat bran medium at 25 °C for 7 days and macerated at the ratio of 500 g of medium incubated *R. solani* per 1 L of distilled water into the mixer. The treated and control plants were held in a lighted chamber (28 °C; photoperiod, 12 h; light intensity, 3000 lux) for 5 days. With CGM caused by *B. cinerea*, the cucumber plants at the first leaf stage (one plants/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1×10^6 spores/mL) of *B. cinerea* incubated on PDA medium at 20 °C for 15 days by leaf spray and then placed in a chamber (20 °C) for 4−5 days. For TLB caused by *P. infestans*, each test solution was sprayed onto tomato plants at the second leaf stage (two plants/pot). The plants were inoculated with a suspension of 1×10^5 zoosporangia/mL made from a 14 day culture of V-8 juice agar medium at 20 °C. They were kept in a chamber (18 °C) for 4 days, and disease ratings were made. For WLR caused by *P. recondita*, wheat plants at the first leaf stage (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg/100 mL of 250 ppm Tween 20) of uredospores collected from the second leaf of wheat and then placed in a moist chamber. One day after inoculation, the plants were held in a growth chamber (20 °C and 70% RH). The

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Table 2. Antifungal Activities of *C. tora* Seed-Derived Materials against Phytopathogenic Fungi[a]

material	concn (g/L)	control values[b] (%)					
		RCB	RSB	CGM	TLB	WLR	BPM
methanol extract	2	0	100	100	100	0	100
	1	0	91	82	75	0	87
hexane fraction	2	0	0	71	0	0	0
	1	0	0	45	0	0	0
chloroform fraction	2	0	100	100	100	0	100
	1	0	95	86	85	0	93
ethyl acetate fraction	2	0	0	0	68	0	85
	1	0	0	0	45	0	62
LSD (0.05)			4.5	8.5	9.6		8.8

[a] Activity is preventive value (%), 100% complete killing, 0% zero killing. [b] RCB, caused by *Py. grisea* on rice; RSB, caused by *R. solani* on rice; CGM, caused by *B. cinerea* on cucumber; TLB, caused by *P. infestans* on tomato; WLR, caused by *P. recondita* on wheat; and BPM, caused by *E. graminis* on barley.

fungicidal activities of the test samples were tested 10 days after inoculation. For BPM caused by *E. graminis*, barley plants with a fully expended first leaf (four plants/pot: φ 7.5 cm) were sprayed with a suspension of the test material. The treated plants were dusted with *E. graminis* conidia formed on leaves of barley. The control effect of test samples on each disease was evaluated with a control value (CV) calculated by the formula CV (%) = [(A − B)/A] × 100, where A and B represent the disease area on the untreated and treated plants, respectively. The LC_{50} values were calculated by probit analysis.

Statistical Analysis. Analysis of variance was performed using the PROC GLM procedure (SAS Institute, Cary, NC). If *P* was less than 0.01, the means were separated with the least significant difference (LSD) test at the *P* = 0.05 level.

RESULTS AND DISCUSSION

During the initial experiments, we observed that a methanolic extract of *C. tora* seeds possessed significant fungicidal activities (100%) against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* at the concentration of 2 g/L. However, this methanolic fraction showed no fungicidal activity against *Py. grisea* and *P. recondite*. Further solvent fractionation showed strong fungicidal activities in the chloroform fraction against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* with 86, 93, 85, and 95% control values, respectively, at the concentration of 1 g/L (**Table 2**). Furthermore, the ethyl acetate fraction had fungicidal activities against *P. infestans* and *E. graminis* with 45 and 62% control values, and the hexane fraction had a fungicidal activity against *B. cinerea* with 45% control value at 1 g/L. However, little fungicidal activity was observed with the butanol and water fractions (not shown).

Because of the strong activity of the chloroform fraction, purification of the biologically active compounds was achieved with silica gel column, Sephadex LH-20 column, Polyclar AT column, Sephadex LH-20 column, and cellulose chromatography, and the isolates were bioassayed. Three active isolates showed potent fungicidal activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* (**Table 3**). The structural determination of the isolates was made by spectroscopic methods including MS and NMR and by direct comparison with authentic reference compounds, and they were characterized as the anthraquinones rhein (compound I), emodin (comound II), and physcion (compound III) (**Figure 1**). These compounds were identified on the basis of the following evidence. Rhein: orange needles from CHCl₃, mp 328−330 °C. IR (KBr): υ_max 3600 (OH), 2200, 1689, 1625, 1605, 1484, 1265, 1187, 1094, 752 cm⁻¹. UV (MeOH) λ_max nm (log ε): 230 (4.6), 260 (4.2), 432 (4.0). ¹H NMR [(CD₃)₂SO]: δ 2.07 (1H, d, *J* = 1.7 Hz, H-1),

Table 3. Antifungal Activities of Components Isolated from *C. tora* Seeds, Three Commercially Available Anthraquinones, and Synthetic Fungicides against Phytopathogenic Fungi[a]

material	concn (g/L)	control values[b](%)					
		RCB	RSB	CGM	TLB	WLR	BPM
emodin	1	0	100	100	81	0	100
	0.5	0	100	85	65	0	100
	0.25	0	76	65	32	0	94
	0.125	0	54	43	0	0	80
	0.0625	0	23	18	0	0	60
	LC_{50} (g/L)		0.102	0.163	0.385		0.046
physcion	1	0	100	92	75	0	100
	0.5	0	90	71	48	0	90
	0.25	0	52	49	12	0	79
	0.125	0	28	25	0	0	65
	0.0625	0	0	0	0	0	49
	LC_{50} (g/L)		0.248	0.263	0.518		0.073
rhein	1	0	83	78	100	0	0
	0.5	0	65	51	100	0	0
	0.25	0	35	34	90	0	0
	0.125	0	23	14	81	0	0
	0.0625	0	0	0	60	0	0
	LC_{50} (g/L)		0.375	0.478	0.047		
aloe-emodin	1	0	100	91	0	0	0
	0.5	0	86	74	0	0	0
	0.25	0	67	47	0	0	0
	0.125	0	36	20	0	0	0
	0.0625	0	11	0	0	0	0
	LC_{50} (g/L)		0.177	0.275			
chlorothalonil[c]	0.05	0	0	0	94	0	0
dichlofluanid	0.05	0	0	91	0	0	0
LSD (0.05)			9.1	10.7	8.4		7.5

[a] Activity is preventive value (%), 100% complete killing, 0% zero killing. [b] RCB, caused by *Py. grisea* on rice; RSB, caused by *R. solani* on rice; CGM, caused by *B. cinerea* on cucumber; TLB, caused by *P. infestans* on tomato; WLR, caused by *P. recondita* on wheat; and BPM, caused by *E. graminis* on barley. [c] Commercial name.
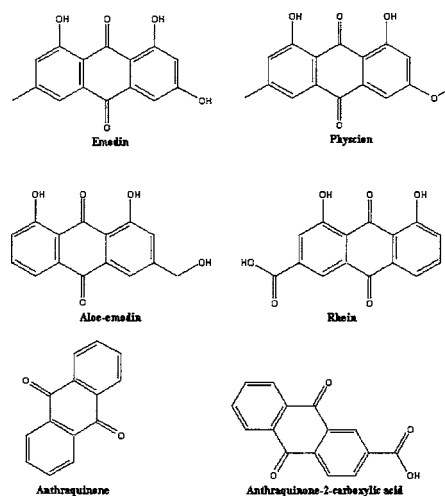
Figure 1. Structure of anthraquinones isolated from *C. tora* seed.

7.37 (1H, d, *J* = 8.2 Hz, H-3), 7.70 (1H, m, H-2), 8.09 (1H, t, *J* = 3.4 Hz, H-10), 11.87 (1H, s, H-7). HREIMS *m/z* 284.2205 (C₁₅H₈O₆). ¹³C NMR [(CD₃)₂SO]: δ 191.3, 181.0, 165.4, 161.4, 161.1, 138.2, 137.6, 133.8, 133.2, 124.6, 124.1, 119.4, 118.8, 118.7, 116.2. Emodin: orange needles from EtOH, mp 260−263 °C. IR (KBr): υ_max 3425(O-H), 1677, 1627 (C=O) cm⁻¹. UV (MeOH) λ_max nm (log ε): 248 (4.11), 262 (4.12), 285 (4.18), 432 (3.92). ¹H NMR [(CD₃)₂CO]: δ 2.46 (3H, s, Ar-CH₃), 6.65 (1H, d, *J* = 2.5 Hz, H-2), 7.12 (1H, br s, H-7), 7.24 (1H, d, *J* = 2.5 Hz, H-4), 7.55 (1H, br s, H-5), 12.05 (1H, s, OH), 12.18 (1H, s, OH). HREIMS *m/z* 270.0517 (C₁₅H₁₀O₅). ¹³C NMR

[(CD$_3$)$_2$CO]: δ 191.9, 182.2, 167.0, 165.9, 161.9, 149.3, 136.6, 134.0, 124.8, 121.2, 114.4, 110.0, 109.7, 108.6, 21.8. Physcion: orange needles from EtOH, mp 207−209 °C. IR (KBr): ν_{max} 3400 (OH), 1630 (C=O) cm^{-1}. UV (MeOH) λ_{max} nm (log ϵ): 223, 254, 265, 287, 434. ^{1}H NMR [(CD$_3$)$_2$CO]: δ 2.46 (3H, s, Ar-CH$_3$), 3.94 (3H, s, Ar-CH$_3$), 6.68 (1H, d, J = 2.5 Hz, H-2), 7.08 (1H, br s, H-7), 7.36 (1H, d, J = 2.5 Hz, H-4), 7.63 (1H, br s, H-5), 12.12 (1H, s, OH), 12.31 (1H, s, OH). HREIMS m/z 284.0708 (C$_{16}$H$_{12}$O$_5$). ^{13}C NMR [(CD$_3$)$_2$CO]: δ 187.0, 166.8, 159.6, 158.5, 142.5, 141.8, 140.7, 123.6, 123.1, 119.8, 118.9, 108.0, 104.7, 56.0, 21.2. The spectroscopic analyses of emodin, rhein, and physcion were found to be the same as those for emodin from *Cassia obtusifolia* (*13*) and rhein and physcion from *Rheum emodi* (*14*).

The fungicidal activities of emodin, rhein, and physcion isolated from *C. tora* seeds against six phytopathogenic fungi when treated with various concentrations were determined in vivo (**Table 3**). Emodin and physcion showed strong to moderate activities against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* at 1, 0.5, and 0.25 g/L, except for WLR caused by *P. recondita* and RCB caused by *Py. grisea*. Rhein exhibited strong fungicidal activities against *B. cinerea*, *P. infestans*, and *R. solani* at 1, 0.5, and 0.25 g/L, except for WLR and BPM caused by *E. graminis* and *P. recondita* and RCB caused by *Py. grisea*. Emodin has an apparent LC$_{50}$ value of approximately 0.102, 0.163, 0.385, and 0.046 g/L against *R. solani*, *B. cinerea*, *P. infestans*, and *E. graminis*, respectively, and physcion has an LC$_{50}$ value of 0.248, 0.263, 0.518, and 0.073 g/L against *R. solani*, *B. cinerea*, *P. infestans*, and *E. graminis*, respectively (**Table 3**). Furthermore, the LC$_{50}$ value of rhein is 0.375, 0.478, and 0.047 g/L against *R. solani*, *B. cinerea*, and *P. infestans*, respectively. This study is the first to report the fungicidal functions of these components isolated from *C. tora* seeds against *B. cineria*, *E. graminis*, *P. infestans*, *P. recondita*, *P. grisea*, and *R. solani*. The *Cassia* species are well-known traditional Chinese medicinal plants. The seeds of the plant have been widely used for the treatment of red and tearing eyes, headache, and dizziness (*15*). They are also used as an essential ingredient in medicine as antiseptic, antidiarrheal, antioxidant, antimicrobial, and antimutagen medicines (*10−12*, *16*). Among the constituents of *C. tora*, anthraquiones constitute a major group of secondary metabolites (*16*). Anthraquinones (aloe-emodin, chrysophanol, physcion, and rhein) from *R. emodi* have been reported to exhibit antifungal activities against *Candida anbicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, and *Aspergillus fumigatus* (*14*). Furthermore, anthraquinones (anthraquinone, anthraflavine, anthrarufin, and quinizarin) exhibited a strong inhibition against *Clostridium perfringens* and *Staphylococcus aureus* and strongly inhibited the mouse liver microsomal conversion of aflatoxin B$_1$ into aflatoxin B$_1$-8,9-epoxide (*16*).

To further examine the fungicidal activity of anthraquinones, three additional structurally related anthraquinones, aloe-emodin, anthraquinone, and anthraquinone-2-carboxylic acid, were determined against the six phytopathogenic fungi (**Figure 1** and **Table 3**). Aloe-emodin revealed strong to moderate fungicidal activities against *B. cinerea* and *R. solani* at 1, 0.5, and 0.25 g/L but did not inhibit the growth of *E. graminis*, *P. infestans*, *P. recondita*, and *Py. grisea*. Aloe-emodin has an apparent LC$_{50}$ value of 0.177 and 0.275 g/L against *R. solani* and *B. cinerea*, respectively. Little or no activity was observed for anthraquinone and anthraquinone-2-carboxylic acid when treated at 1 g/L (not shown). In the current study, these results indicated that the aromatic hydroxyls are probably important moieties for anti-

fungal actions, given that the four compounds (aloe-emodin, emodin, physcion, and rhein) have at least two such groups whereas anthraquinone and anthraquinone-2-carboxylic acid lack them. Because of the fungicidal activities of rhein, emodin, and physcion against *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani*, the compounds were compared with the synthetic fungicides chlorothalonil and dichlofluanid (**Table 3**). A potent fungicidal activity was observed with chlorothalonil against *P. infestans* at 0.05 g/L and dichlofluanid against *B. cinerea* at 0.05 g/L, whereas no fungicidal activity was produced for chlorothalonil against *B. cineria*, *P. grisea*, *R. solani*, and *P. recondita* or for dichlofluanid against *P. grisea*, *P. infestans*, *P. recondita*, and *R. solani*. In this study, although the fungicidal activity of rhein, emodin, and physcion is less than that of the synthetic fungicides used, rhein, emodin, and physcion may be useful as leads developing new types of fungicides for controlling *B. cinerea*, *E. graminis*, *P. infestans*, and *R. solani* on crops.

Certain plant extracts and phytochemicals act in many ways on various types of disease-causing agents and may be applied to the crop in the same way as other agricultural chemicals. They are being considered as potential alternatives for synthetic fungicides (*17*, *18*) or lead compounds for new classes of synthetic fungicides such as podoblastin produced by *Podophyllum peltatum* (*19*, *20*).

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