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Research Article

Antifungal effects of *Paeonia lactiflora* seed extracts

Kyung-Tack Kim[1]*, Dong Gu Lee[2], Sang Yoon Choi[1]

[1]*Food Research Division, Korea Food Research Institute, Wanju 55365, Korea*
[2]*Jabez-Bio Company, Andong 36611, Korea*

Abstract *Paeonia lactiflora* is a plant resources widely distributed in Asia. The root of *Paeonia lactiflora* have been used to traditional herbal medicine for blood stasis syndrome in Korea. Until now, many studies have been conducted on the root of *Paeonia lactiflora,* but studies on seed are insufficient. This study was carried out to determine the inhibitory effect of *Paeonia lactiflora* seed extract against the phytopathogenic fungi *Alternaria panax, Botrytis cinerea, Colletotrichum acutatum, Cylindrocarpon destructans, Colletotrichum gloseosporiades, Phytophthora cactorum*, and *Pythium ultimum*. The results demonstrated that the seed extract showed potent antifungal effects against *Alternaria panax*, *Colletotrichum acutatum*, *Cylindrocarpon destructans*, *Colletotrichum gloseosporiades*, *Phytophthora cactorum*, and *Pythium ultimum. Especially*, the *Paeonia lactiflora* seed extract showed the highest antifungal activity against *Pythium ultimum and Phytophthora cactorum,* and the antifungal activity was not affected by heat treatment. Therefore, the antifungal component of the *Paeonia lactiflora* seed extract was concluded thermostable and it is suggested that the *Paeonia lacflora* seed extract has stability for antifungal activity maintaining. These results suggested that *Paeonia lacflora* seed extract may be useful as a natural antifungal agent to prevent plant diseases.

Keywords *Paeonia lactiflora*, seed, antifungal, phytopathogenic fungi

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***Corresponding author**
Kyung-Tack Kim
Tel: +82-63-219-9096
E-mail: tack@kfri.re.kr

1. Introduction

Insects, weeds, and phytopathogenic fungi cause significant damage to agriculture, homes, and gardens. If these are not controlled systematically, an estimated 40% of a typical crop is lost preharvest and an additional 20% is lost postharvest (Pimentel et al., 1992). Synthetic pesticides have successfully controlled numerous agricultural pests; however, the development of resistance in pests to current pesticides and high costs for the development of new chemicals limit the efficiency of synthetic pesticides. Furthermore, residues of synthetic pesticides in agricultural products threaten human health and soil and water ecosystems (Di et al., 2022; Liu et al., 2021; Wei et al., 2021). Thus, natural chemicals produced by plants (plant extracts or phytochemicals), fungi, and bacteria, which constitute a rich source of bioactive substances, may be an alternative to the currently used pesticides for controlling insect pests, phytopathogenic fungi, and mosquito larvae

(Kalhoro et al., 2022; Seepe et al., 2021; Swain, 1997; Wink, 1993).

The genus *Paeonia* (family Paeoniaceae) is distributed throughout China, India, Japan, Korea, and Vietnam (Bich et al., 2004). The roots of *Paeonia lactiflora* Pall. have been frequently prescribed as an alternative medicine to enhance blood circulation and inhibit inflammation (Cameron and Cruickshank, 2007; Perrin et al., 2007; Zhang et al., 2020). However, very few phytochemical studies are available on seeds. Recently, resveratrol and its derivatives, possessing anti-carcinogenic, anti-mutagenic, and anti-oxidative activities, were isolated from *Paeonia* seeds (Kim et al., 2002a; Kim et al., 2002b; Wu et al., 2022). The objective of this study was to investigate fungicidal effects of the seed extract of *P. lactiflora* against *Alternaria panax*, *Botrytis cinerea*, *Colletotrichum acutatum*, *Cylindrocarpon destructans*, *Colletotrichum gloseosporiades*, *Phytophthora cactorum*, and *Pythium ultimum* and test the potential of this species as an effective and environment-friendly alternative.

2. Materials and methods

2.1. Plant

Seeds of *Paeonia lactiflora* were obtained from Euiseong herb experimental (Euiseong-gun, Kyungpook, Korea). 1.5 kg of the seeds was subjected to extraction with 70% methanol by shaking for 5 days, and it was subjected to further extraction three times for 12 h each. After filtration through Whatman No.2 filter paper, the extract was concentrated at 40℃ using a rotary evaporator.

2.2. Fungal strains

Seven phytopathogenic fungi (*A. panax*, *B. cinerea*, *C. acutatum*, *C. destructans*, *C. gloseosporiades*, *P. cactorum*, and *P. ultimum*) were donated by Korean Agricultural Culture Collection (KACC).

2.3. Paper disc assay

Potato dextrose agar (PDA) was accurately weighed and dissolved in distilled water, kept in an Erlenmeyer flask, plugged with cotton, and sterilized. After sterilization, approximately 20 mL of the agar medium was poured into sterilized Petri dishes (9 cm diameter), allowed to cool and solidify, and inoculated. Antifungal assay was performed on the solid media by disc diffusion method (Duru et al, 2003). Eight sterile Whatman paper discs (8 mm diameter) were pierced in the agar plates, equidistant and near the border, and separately loaded with 50 μL solution containing 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, and 2.5 mg of the extract each, and then dried at room temperature for 30 min.

Discs of fungal inocula, 8 mm in diameter, were collected from pre-grown cultures of each of the fungal strains to be tested and placed upside down in the center of the Petri dishes containing agar and extract discs. The plates were incubated at 28℃ for 6 days. The diameter of the mycelial area was measured in each dish. An average of three measurements of diameter was considered.

2.4. Percent inhibition of mycelial growth assay

Percent inhibition of mycelial growth (PIMG) assay was conducted for the quantitative analysis of the antifungal effect of *P. lactiflora* seed extract. Six mixtures of PDA with seed extract were prepared at concentrations of 10, 50, 100, 200, 300, and 400 μg of the seed extract/mL, respectively. After autoclaving at 121℃ for 15 min, these media were poured into separate sterilized Petri dishes. Agar discs (8 mm) were taken from the fungal cultures and placed in the center of each of the

Petri plates. Similarly, for control, fungal agar discs (8 mm diameter) were placed on fresh PDA plates without seed extracts. All cultures were incubated at 28℃ for 6 days. Inhibitory activity was assessed by measuring the radial growth of mycelia on the treated media (R_2) and radial growth on the control plates (R_1), and using the formula:

$$PIMG = \{(R_1 - R_2) / R_1\} \times 100.$$

2.5. Measurement of minimal inhibitory concentration

The minimal inhibitory concentration (MIC) of the antifungal agents against various fungi was determined using broth micro-dilution method, as the lowest concentration at which no fungal growth was observed. 19.6 mL of potato dextrose broth medium was dispensed into 100 mL Erlenmeyer flasks and autoclaved at 121℃ for 15 min. 200 μL of different doses (final concentration 2.5-500 μg/mL) of the extract were diluted in methanol and mixed separately into the liquified PDBs. The fungal inoculum (200 μL suspension, 10^7 spores/mL concentration) was added to each of the Erlenmeyer flasks. The tests were first performed using a high concentration of the extract, which was further diluted until no inhibitory activity was observed. Media without extract treatment were used as controls. The fungal cultures were incubated at 28℃ for 7 days and observed for growth inhibition.

2.6. Mycelial growth assay

Flasks with mycelial growth were filtered through Whatman GF/C filter paper and washed with distilled water. The mycelia were placed on pre-weighed filter papers, were allowed to dry at 60℃ overnight, and then weighed. Growth inhibition based on dry weight was calculated using the following formula;

Growth inhibition = Control weight - Sample weight

2.7. Thermal stability measurement

Thermal stability of the antifungal component in the extract was determined by heating the extract at 121℃ for 15 min and loading to 8 mm filter paper discs at 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2, and 2.5 mg of extract/disc concentrations and dried at room temperature for 30 min. These discs were placed on PDA plates and incubated at 28℃ for six days. The diameter of mycelia in each dish was measured. An average of three measurements of diameter was considered. *P. cactorum* was used for the thermal stability measurement.

2.8. Statistical analysis

All data are expressed as the mean±standard error (SE). Statistical significance was analyzed using Student's t-test. Statistical significance was maximum at $p<0.05$ vs. the control (extract-untreated group).

3. Results and discussion

3.1. Antifungal effects

Antifungal activity of *P. lactiflora* seed extract was evaluated against seven phytopathogenic fungi using disc diffusion assay. Extract was added to discs at 0.05, 0.1, 0.25, 0.5, 1, 1.5, 2 and 2.5 mg/disc concentrations, respectively. Table 1 presents the results of the assay. An increase in the inhibition zone was observed with the increase in concentration of the extract. The seed extract showed antifungal effects against all seven phytopathogenic fungi at concentrations 1-2.5 mg/disc. Further, antifungal activity was the highest against *P. cactorum* and lowest against *B. cinerea*.

To evaluate PIMG, the extract (concentrations of 0, 10, 50, 100, 200, 300, and 400 μg/mL) was added

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Table 1. Antifungal activity of *Paeonia lactiflora* seed extracts on seven phytopathogenic fungi

Concentrations (mg/disc)	Clear zone (mm)[1] on plate			
	Fungi			
	Alternaria panax	*Botrytis cinerea*	*Colletotrichum acutatum*	*Cylindrocarpon destructans*
0.05	–[2]	–	–	–
0.1	–	–	–	–
0.25	5.23±1.74[3]	–	–	3.64±2.10
0.5	10.14±1.34	–	–	3.53±0.92
1	12.37±1.08	2.21±1.28	8.38±1.18	10.13±0.87
1.5	16.99±1.45	3.97±0.81	11.04±1.33	11.78±1.15
2	18.85±1.01	5.29±0.28	15.30±1.89	15.48±1.10
2.5	20.32±1.41	6.45±0.52	16.64±0.58	15.57±1.21

Concentrations (mg/disc)	Clear zone (mm) on plate		
	Fungi		
	Colletotrichum gloseosporiades	*Phytophthora cactorum*	*Pythium ultimum*
0.05	–	–	–
0.1	4.82±0.79	6.84±1.41	3.35±0.90
0.25	5.18±0.91	10.56±1.11	5.64±0.66
0.5	5.81±0.85	14.23±1.18	7.07±0.76
1	6.83±0.63	17.00±0.88	7.94±0.91
1.5	7.45±0.66	18.30±0.94	9.64±0.64
2	9.14±1.02	21.49±1.26	11.74±0.97
2.5	10.68±1.03	21.61±1.03	12.59±0.83

[1]Zone of inhibition showing no growth surrounding 8 mm filter paper disc saturated with 50 μL of test sample.
[2]No inhibition zone was formed.
[3]Standard error of three experiments.

to separate PDA mixtures and inoculated. The extract showed antifungal effects against all seven phytopathogenic fungi at concentrations 50-400 μg/mL. PIMG was the highest (88.39%) for *P. ultimum* at 400 μg/mL (Fig. 1 and Fig. 2).

The MIC is the lowest concentration at which the growth of target phytopathogenic fungi is inhibited. We examined the inhibitory action of the extract at 2.5-500 μg/mL concentrations against the seven phytopathogenic fungi (Table 2). The seed extract showed strong antifungal activity at 20-500 μg/mL concentrations. The MIC of the seed extract was observed at 20 μg/mL for *P. ultimum* and *P. cactorum*, 100 μg/mL for *A. panax*, 200 μg/mL for *C. destructans* and *C. gloseosporiades*, 400 μg/mL for *C. acutatum*, and 500 μg/mL for *B. cinerea*. Thus, the strongest inhibitory activity was observed against *P. ultimum* and *P. cactorum*. The MIC values suggest that cell growth inhibition was mediated by fungicidal action of the seed extract.

In this study, the inhibitory effect against fungi of *Paeonia lacflora* seed extract evaluated. It can be suggested that *Paeonia lacflora* seed extract contain potential antifungal compounds and the obtained results may also be useful for finding active substances. Therefore, active chemical

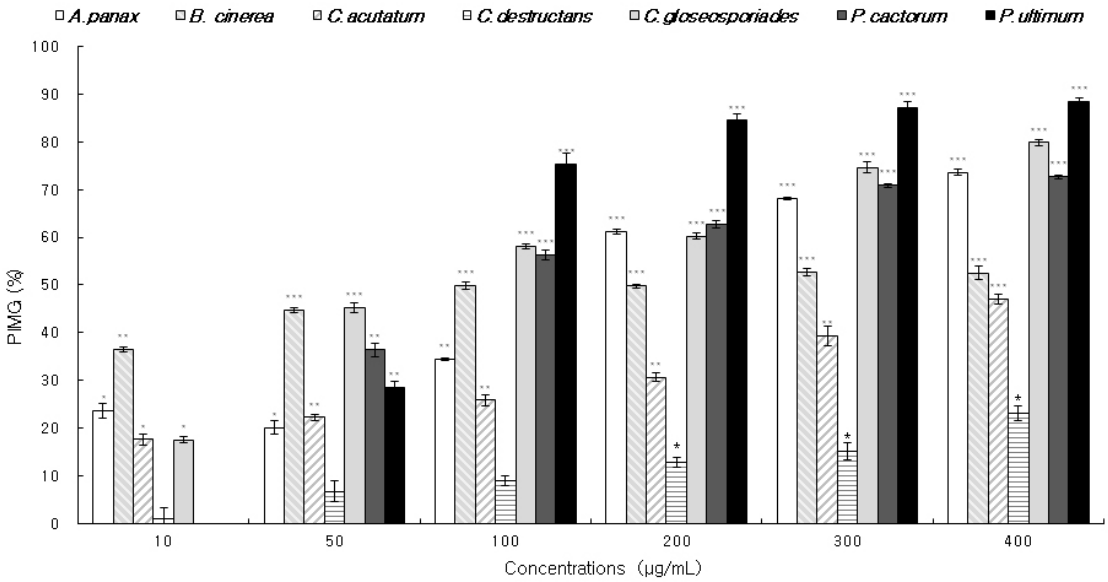

Fig. 1. Inhibitory effects of *Paeonia lactiflora* seed extracts on the mycelial growth of seven phytopathogenic fungi. Percent inhibition of mycelia growth (PIMG) was measured after 6 days of incubation at 28℃. $^{*}p<0.05$, $^{**}p<0.01$, and $^{***}p<0.001$ vs. control (extract-untreated group).

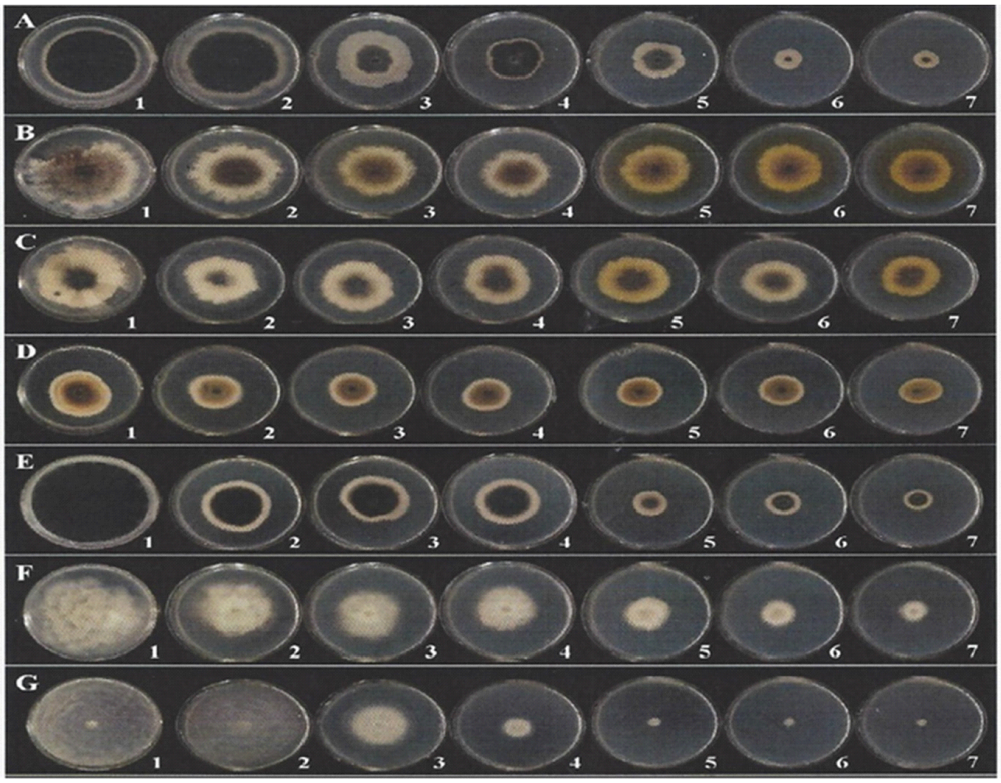

Fig. 2. Inhibitory effects of *Paeonia lactiflora* seed extracts on the mycelia growth of *Alternaria panax* (A), *Botrytis cinerea* (B), *Colletotrichum acutatum* (C), *Cylindrocarpon destructans* (D), *Colletotrichum gloseosporiades* (E), *Phytophthora cactorum* (F), and *Pythium ultimum* (G). 1 indicates control (fresh PDA). 2-7 contain 10, 50, 100, 200, 300, and 400 μg of extract/mL of media, respectively. Percent inhibition of mycelial growth (PIMG) was calculated after 6 days of incubation at 28℃.

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Table 2. The minimal inhibitory concentration (MIC) of *Paeonia lactiflora* seed extracts on seven phytopathogenic fungi

Concentrations (μg/mL)	Fungi			
	Alternaria panax	*Botrytis cinerea*	*Colletotrichum acutatum*	*Cylindrocarpon destructans*
Control	0.04[1)]±0.01[2)]	0.09±0.00	0.10±0.00	0.10±0.01
2.5	0.03±0.01	0.07±0.00	0.08±0.00	0.05±0.00
5	0.03±0.00	0.07±0.00	0.08±0.00	0.05±0.00
10	0.03±0.01	0.07±0.00	0.08±0.00	0.04±0.00
20	0.02±0.00	0.06±0.00	0.08±0.00	0.04±0.00
50	0.01±0.00	0.04±0.00	0.07±0.00	0.03±0.00
100	0.00±0.00	0.04±0.01	0.07±0.00	0.02±0.01
150	0.00±0.00	0.04±0.01	0.06±0.00	0.01±0.00
200	0.00±0.00	0.04±0.01	0.05±0.01	0.00±0.00
300	0.00±0.00	0.03±0.00	0.05±0.00	0.00±0.00
400	0.00±0.00	0.03±0.00	0.00±0.00	0.00±0.00
500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
MIC[3)]	100	500	400	200

Concentrations (μg/mL)	Fungi		
	Colletotrichum gloseosporiades	*Phytophthora cactorum*	*Pythium ultimum*
Control	0.07±0.00	0.06±0.00	0.09±0.01
2.5	0.06±0.00	0.02±0.00	0.06±0.00
5	0.06±0.00	0.01±0.00	0.04±0.01
10	0.06±0.00	0.01±0.00	0.03±0.00
20	0.06±0.00	0.00±0.00	0.00±0.00
50	0.06±0.00	0.00±0.00	0.00±0.00
100	0.06±0.00	0.00±0.00	0.00±0.00
150	0.01±0.00	0.00±0.00	0.00±0.00
200	0.00±0.00	0.00±0.00	0.00±0.00
300	0.00±0.00	0.00±0.00	0.00±0.00
400	0.00±0.00	0.00±0.00	0.00±0.00
500	0.00±0.00	0.00±0.00	0.00±0.00
MIC	200	20	20

[1)]Dry weight (g) of mycelia.
[2)]Standard error of three experiments.
[3)]MIC, minimal inhibitory concentration (μg/mL).

compound of *Paeonia lacflora* seed extract will be further investigated in further studies.

3.2. Thermal stability of antifungal component in the extract

The antifungal activity of the extract against *P. cactorum* (cultured for 6 days) in relation to temperature is shown in Table 3. The inhibitory effect of the extract (in terms of inhibition zone) without heat treatment was similar to that of the extract heated at 121℃ for 15 min, implying that the antifungal activity of the extract was not

Table 3. Thermal stability of *Paeonia lactiflora* seed extract

Concentrations (mg/disc)	Clear zone (mm)[1] on plate	
	Phytophthora cactorum	
	No heat	121℃, 15 min
0.05	–[2]	–
0.1	–	–
0.25	9.31±0.32[3]	8.23±0.26
0.5	11.86±0.34	10.13±0.58
1	14.33±0.98	12.75±1.13
1.5	16.14±0.56	14.07±0.88
2	17.86±1.03	16.24±0.78
2.5	18.80±1.01	17.41±0.75

[1]Zone of inhibition showing no growth surrounding 8 mm filter paper disc saturated with 50 μL of test sample.
[2]No inhibition zone was formed.
[3]Standard error of three experiments.

affected by the increase in temperature. Therefore, the antifungal component of the seed extract was concluded thermostable and it is suggested that the *Paeonia lacflora* seed extract has stability for antifungal activity maintaining.

4. Conclusions

In this study, *P. lactiflora* seed extract was used to evaluate its inhibitory effect against selected phytopathogenic fungi. The seed extract showed potent antifungal activity against *A. panax, C. acutatum, C. destructans, C. gloseosporiades, P. cactorum* and *P. ultimum*. Further, antifungal activity was the highest against *P. cactorum* and *P. ultimum*. These results suggest that *P. lactiflora* seed extract may be effective for management of numerous plant diseases by reducing the effects of pathogenic fungi.

Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Kim KT, Lee DG. Data curation: Lee DG, Choi SY. Formal analysis: Kim KT, Lee DG. Methodology: Kim KT, Lee DG. Validation: Kim KT, Lee DG. Writing - original draft: Kim KT, Lee DG. Writing - review & editing: Kim KT.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Kyung-Tack Kim (First & Corresponding author)
https://orcid.org/0000-0002-5753-9870
Dong Gu Lee
https://orcid.org/0000-0002-7333-4912
Sang Yoon Choi
https://orcid.org/0000-0001-8712-6877

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