Emirates Journal of Food and Agriculture. 2016. 28(11): 818-822
doi: 10.9755/ejfa.2015-12-1077
http://www.ejfa.me/

SHORT COMMUNICATION

Antifungal activity of *Araliae Continentalis Radix* extract on rice sheath blight

Tae-Seok Oh, Youn-Jin Park, Chang-Ho Kim, Yong-Koo Cho, Ki-sung Kwon, Tae-Kwon Kim, Myoung-Jun Jang*

Department of Plant Resources, Kongju National University, College of Industrial Sciences, Yesan, Republic of Korea, South Korea

ABSTRACT

Rice sheath blight is caused by *Rhizoctonia solan*, a plant pathogenic fungus that is spread by insect pests as well as splashing rain and plant contact, causing the most serious damage globally with the second largest cost for antifungal control. The *Aralia Continentalis Radix* extract has an antifungal activity against *Rhizoctonia solan*, and it was presumed that the ent-Pimara-8(14), 15-diene-19-oic acid contained in *Aralia Continentalis Radix* is the chemical constituent of the antifungal metabolite. It was confirmed that the *Aralia Continentalis Radix* extract can be used as a control agent against rice sheath blight. The damage caused by rice sheath blight was decreased to less than 16% when the *Aralia Continentalis Radix* extract (125 mg.L^{-1} or more) was sprayed onto rice plants in a disaster prevention experiment in a field afflicted by rice sheath blight.

Keywords: Antifungal activity; Pesticide; *Aralia Continentalis Radix*; Antifungal agent; Natural substance

INTRODUCTION

Rice sheath blight is caused by *Rhizoctonia solan*, a plant pathogenic fungus that is spread by insect pests as well as splashing rain and plant contact, causing the most serious damage globally with the second largest cost for antifungal control. When rice sheath blight occurs, the leaf color of rice turns greyish white, and the transport of nutrients as well as moisture is blocked resulting in physiological impairment in which the infected leaves dry out and the plant dies. In serious cases, lodging may incur causing large economic losses (Lee et al, 2010).

Rhizoctonia solan, which is the fungal pathogen of rice sheath blight, has a wide range of hosts; however, there are few breeds of rice with resistance to it causing large losses in labor and costs for antifungal control (Park et al, 2008).

When comparing the yearly prevalence of sheath blight, 2001 had the lowest prevalence nationwide for which the percentage of top lesion height vs plant height (PLH) was 10.2% whereas 2007 had the highest prevalence with a percentage of 21.4% for the top lesion height vs plant height (PLH). Sheath blight is known to be have prevalence in conditions with high temperatures ranging from 23-35°C and high humidity ≥ 96% and only small periods of sunshine (Endo, 1935; Yoshimura, 1995).

In the past, synthetic anti-microbial agents have been used a lot to control frequently occurring plant diseases like rice sheath blight, but lately, social concern for the use of synthetic anti-microbial agents has been increasing in connection to fungicide-related safety issues for agricultural products and environmental contamination. Consequently, the needs for biologica l pesticides for insect pests and eco-friendly materials for environmentally friendly agriculture are rapidly increasing. (Burpee and Goulty, 1984; Yoshie et al., 1993).

Additionally, the use of extracted antifungal substances from natural materials such as *Araliae Continentalis Radix* is increasing. *Aralia Continentalis Radix* is a perennial plant belonging to the *Aralia elata* family. It is used as an oriental medicine with efficacy for anti-inflammation and anti-platelet aggregation (Han et al., 1983). The continentalic acid and sterol compound contained in *Araliae Continentalis*

***Corresponding author:**
Myoung-Jun Jang, Department of Plant Resources, Kongju National University, College of Industrial Sciences, Yesan, Republic of Korea, South Korea. Mobile: +82-41-330-1200, Fax: +82-41-330-1209. E-mail: plant119@kongju.ac.kr

Received: 09 December 2015; **Revised:** 18 September 2016; **Accepted:** 21 September 2016; **Published Online:** 30 October 2016

Radix have been reported as a substance with high antifungal activity recently (Han, 2004).

To this end, this clinical study examined the source material and investigated the use of *Araliae Continentalis Radix* for the development of an eco-friendly antifungal agent for rice sheath blight.

MATERIALS AND METHODS

Extraction of the antifungal substance

We purchased *Araliae Continentalis Radix* locally grown from an oriental medicinal material vendor located in Dang Jin Si, Chung Cheong Nam Do, Korea and used it as the test material. The raw materials were crushed with a grinding mill (HMF-34 0, Hanil, Korea) and then used as the sample for the extraction. Ethanol was used as the extracting solvent. We mixed the powder specimen of *Araliae Continentalis Radix* and ethanol at a ratio of 1:5 (w/v) and performed an agitated extraction at room temperature for 24 hours. The extracted material was filtered with filtration paper (Whatman, No.2) followed by decompressive concentration (Vacuum Filtration) in a water bath at 45°C in a rotary evaporator (N-1000, Eyela, Japan). Then, we suspended the *Araliae Continentalis Radix* extract by adding 1 L of distilled water and fractionated the extract with chloroform followed by ethyl acetate then n-butanol and finally water obtaining 5.51 g of chloroform, 4.24 g of ethyl acetate, 7.85 g of n-butanol, and 10.92 g of water through inspissation. After decompressive concentration of the ethyl acetate layer which showed a strong inhibition activity, we performed silica gel column chromatography with the solvents CHCl3-MeOH (100:1 to 1:2) and divided it into 6 fractions in order to isolate the antifungal substance. Among those 6 fractions, the No. 5 fraction showed a strong antifungal activity. Therefore, to isolate the active substance from the 5th fraction, we performed silica gel column chromatography with the solvents hexane-acetone (20:1 to 10:1) and obtained compounds 1 (130 mg) and 2 (20 mg). Then, these compounds were identified as ent-Pimara-8(14),15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid through MS and NMR data analyses(supplemental data).

Antifungal activity of the *Araliae Continentalis Radix* extract

We used *Rhizoctonia solan* Kuhn AG-1(IA)(KACC: 40106) supplied by the KACC (Korea Agricultural Culture Collection) as a publicly announced strain of rice sheath blight. To investigate the antifungal activity of the *Araliae Continentalis Radix* extract, we first mixed PDA media with the extract for final concentrations of 62.5, 125, 250 and 500 mg. L-1 before the media hardened when the media was made. Then, we put 10 ml each of the mixture into 9 cm-diameter petri dishes, and after checking for solidification, we took a mycelia disc (5 mm diameter) of sheath blight and placed it onto the center of the medium. The mycelia was cultured at 25°C, and the mycelia growth rate was measured to determine the antifungal activity of the *Araliae Continentalis Radix* extract. As a control, we used Hexaconazole Emulsion after mixing it with the *Araliae Continentalis Radix* extract at the same concentration and compared the antifungal actions using the same method mentioned above.

Antifungal activity assay of ent-pimara-8(14),15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid

After cultivating *Rhizoctonia solan* Kuhn AG-1(IA), the concentration of the mycelia (1×104 piece/mL) was confirmed with a Haematocytometer. Then, the plate medium (test sample) was prepared by smearing the cultured *Rhizoctonia solan* Kuhn AG-1(IA) onto the PDA medium with a spread stick. The ent-Pimara-8(14),15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid were prepared so that their final concentrations were 62.5, 125, 250, and 500 mg. L-1. Each acid was absorbed onto a 8 mm disc paper, and the disc papers were completely dried. The dried discs were placed onto the plates containing the PDA medium and the *Rhizoctonia solan* Kuhn AG-1(IA) and incubated in a 25°C incubator. The antifungal activity for each concentration of the acids was compared by measuring the clear zone generated around the disc.

Test of antifungal control on rice sheath blight for packaging

The testing for packaging fungicide control for rice sheath blight was implemented at the Disease and Insect Pests Observational Plot of the Agricultural Technology Center in Seocheon-gun, Choong Chung Nam-do on August 1, 2013. The selected specimen was Saenuri. A foliar spray of the fungicide was done twice on August 1 and August 7 for each concentration (62.5, 125 and 250mg. L-1) of the *Araliae Continentalis Radix* extract.

Disease and insect pest observational plot

As for control, after spraying a Hexaconazole emulsion at the same levels, we calculated the damage level in accordance with the Disease and Insect Pest Investigation Standards of rice sheath blight on August 14, 2013 based on the research, investigation and analysis criteria for agricultural science technology. The damage rate was calculated as follows:

$$\text{Damage Rate } (\%) = (3n_1 + 2n_2 + 1n_3/3\,N) \times 100,$$

where N is the number of invested tiller, n_2 the number of diseased tiller up to the leaves, n_1 the number of diseased

tiller up to the branch leaves, and n_3 the number of diseased tiller (NDT) up to the third leaves.

All statistical analyses were done with the Duncan test using SAS 8.0 (Statistical Analysis System).

RESULTS AND DISCUSSION

Antifungal activity of the *Araliae Continentalis Radix* extract on *Rhizoctonia solan* Kuhn AG-1(IA)

Araliae Continentalis Radix was reported to have antifungal effects on pathogenic microbes and some plant pathogenic fungi(Oh et al., 2013) in their study reported that *Araliae Continentalis Radix* extract has a high level of antifungal activity against *Pyricularia grisea*, and Han (2004) also reported that *Araliae Continentalis Radix* Extract has an antimicrobial activity against gram-positive bacilli. Because *Araliae Continentalis Radix* is known to have antimicrobial and antifungal activities, we tested the antifungal activity of the *Araliae Continentalis Radix* Extract against Rhizoctonia solan Kuhn AG-1(IA), and the results are shown in Table 1 and Fig. 1.

As for the untreated petri plates, the hyphae of the sheath blight grew to the extent that they almost covered the entire surface of the 9 cm petri dish; however, in the petri dish in which the control and extract were mixed together, the growth level of the hyphae was notably lower compared to the untreated petri plates.

Table 1: Effectiveness of the antifungal activity of the *Araliae Continentalis Radix* extract at various concentrations against *Rhizoctonia solan* Kuhn AG-1(IA)

Treatment ($mg.L^{-1}$)	Growth diameter of hypha (mm)	
	Control	Extract
62.5	12±0.4a	24±1.5a
125	9±1.3b	17±2.1c
250	11±0.9a	21±3.4b
500	10±1.7b*	18±1.1c

*Means followed by the same letter in the same column is not significantly different ($p<0.05$)

For the control, the hyphae of the sheath blight could not grow at all, and the diameter of the hyphae was10 mm for every treatment concentration. The hyphae had a growth diameter of 17 mm when the extract concentration was 125 mg. L-1 which was the smallest diameter of growth. Moreover, at extract concentrations of 500 mg. L-1 and 250 mg. L-1, the growth diameters of the hyphae were 18 mm and 21 mm respectively, showing no large difference. In addition, at the low extract concentration level of 62.5 mg. L-1, the growth diameter was 24 mm. The results show that the extract has antifungal activities against sheath blight (Table 1).

From the aforementioned results, it was possible to confirm that *Araliae Continentalis Radix* has an antifungal activity against sheath blight which was also confirmed by extracting ent-Pimara-8(14),15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid from the *Araliae Continentalis Radix* extract. ent-Pimara-8(14), 15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid have already been reported to have a high level of antimicrobial activity against gram-positive bacillus(Han et al., 2004), but its antifungal activity against sheath blight has not been reported until now.

When we checked the number of colonies that formed after smearing Rhizoctonia solan Kuhn AG-1(IA) onto the medium, the untreated petri plate had so many colonies to the extent that accurate counting of the colonies was impossible, while the control showed such a high level of antifungal activity to the extent that it was not possible to check any of the colonies.

ent-Pimara-8(14), 15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid extracted from the *Araliae Continentalis Radix* extract had a lower level of antifungal activity than that of the Hexaconazole emulsion, which was used as a control, but still had a higher antifungal activity than the untreated petri plate (Table 2).

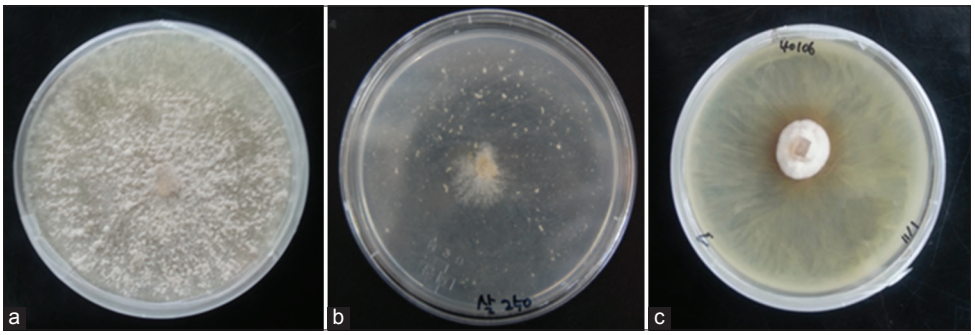

Fig 1. Antifungal activity of the *Araliae Continentalis Radix* extract against rhizoctonia solan Kuhn AG-1(IA) and the control plate after 7 days from strain inoculation. (a: Untreated control plate, b: Control plate (250 mg•L-1), c: Extract of *Araliae Continentalis Radix* (125 mg•L-1)).

Table 2: The effectiveness of the antifungal activity of the organic acids extracted from *Araliae Continentalis Radix* against *Rhizoctonia solan* Kuhn AG-1(IA)

Treatment (mg. L^{-1})	Clear zone (mm)	
	PA*	KA**
62.5	13±0.8a	11±1.1b
125	15±0.4a	12±0.9b
250	13±0.5a	11±0.5b
500	14±1.1a	14±0.6a

*Ent-Pimara-8 (14), 15-diene-19-oic acid, **Ent-Kaur-16-en-19-oic acid

The ent-Pimara-8(14), 15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid have high antimicrobial activity against Gram-positive bacteria; however, their antifungal activity against sheath blight has not been reported until now (Han et al., 2004).

In the case of the ent-Pimara-8(14), 15-diene-19-oic acid, the largest clear zone of 15 mm was formed at a concentration level of 250mg. L-1, and clear zones of 13-15 mm were also confirmed at the different concentrations. In addition, in the case of the ent-Kaur-16-en-19-oic acid, the antifungal activity was observed for all the concentrations, and clear zones of 12 mm and 14 mm were confirmed at concentration levels of 500 mg. L-1 and 250 mg. L-1, and a clear zone of 11 mm was observed for both concentrations of 125 mg. L-1 and 62.5 mg. L-1 (Table 2).

Thus, it was confirmed that ent-Pimara-8(14), 15-diene-19-oic acid and ent-Kaur-16-en-19-oic acid have antifungal activity from the inhibition of growth of Rhizoctonia solan Kuhn AG-1(IA) which is the causative organism of the rice sheath blight (Fig. 2).

Packaging test

As for the weather conditions on the day of the packaging microbicide test for rice sheath blight implemented at the disease and insect pest observational field plot of the Agricultural Technology Center in Seocheon-gun, Choong Chung Nam-do on August 1, 2013, the average temperature from the start of the test (August 1, 2013) until the final test day (August 14, 2013) was 28.5°C. This means a suitable temperature was maintained for the prevalence of rice sheath blight. The damage rate of the untreated plot was 24% showing that suitable packaging conditions had been formed to carry out the test for the antifungal action. The results of the packaging test is shown in Fig. 3.

The damage rates of the control plots on the final test day, August 14, 2013, were all ≤ 13% for all treatment conditions. The lowest damage rate was 9% at 250 mg. L-1, and it was 13% for both concentrations at 125 mg. L-1 and 62.5 mg. L-1, showing a statistically significant difference.

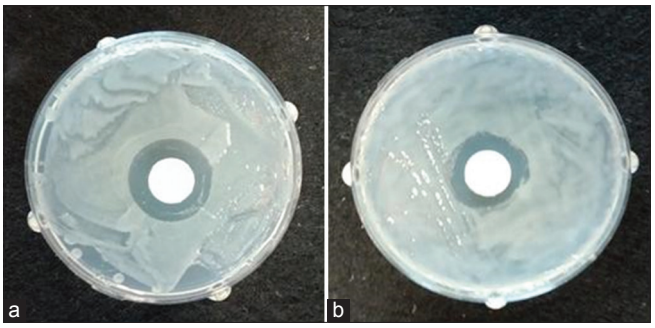

Fig 2. Antifungal Activity of the Organic Acids from the *Araliae Continentalis Radix* Extract against *Rhizoctonia solan* Kuhn AG-1(IA). (a: ent-Pimara-8(14),15-diene-19-oic acid, 250 mg•L-1; b: ent-Kaur-16-en-19-oic acid, 500 mg•L-1).

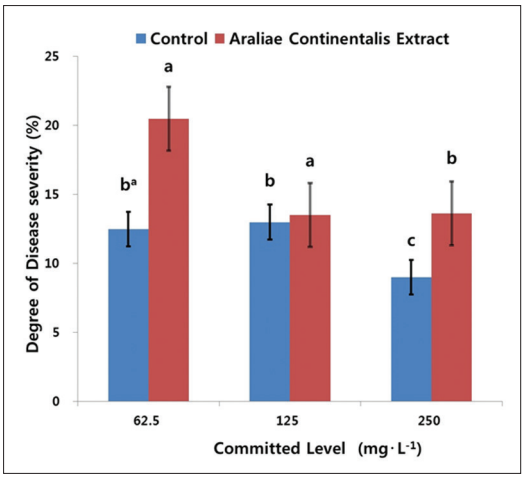

Fig 3. Effects of packaged insect pest of araliae continentalis extract for the rice sheath blight against the control pesticide.

In the case of the *Araliae Continentalis Radix* extract, it had a damage rate of 14% at 250 mg. L-1 showing a lower antifungal effect than that of the control plots; however, no statistically significant difference was identified when compared with the control plot at 125 mg. L-1. Nevertheless, the damage rate was 16% at a concentration level of 125 mg. L-1 with no statistically significant difference; however, the damage rate was high at 21% for 62.5 mg. L-1. Thus, it was confirmed that if using the extract at a concentration ≤ 125 mg. L-1, the extract could not control rice sheath blight effectively.

ACKNOWLEDGEMENT

This work was supported by a research grant from Kongju National University in 2015.

Authors' contribution

Tae Seok Oh and Youn Jin Park were the main researchers of this manuscript. Chang ho Kim, participated in the laboratory experimentation,Young Koo Cho, Ki-sung Kwon and Tae-Kwon Kim performed the field

experimentation, Myung Jun Jang contributed significantly to the research effort and writing, editing specific sections and approved the final version of the manuscript.

REFERENCES

Lee, Y. H., D. S. Ra, W. H. Yeh, H. W. Chii, I. S. Myung, S. W. Lee, Y. H. Lee, S. S. Han and H. S. Shim. 2010. Survey of major disease incidence of rice in Korea during 1999-2008. Res. Plant Dis. 16: 183-190.

Park, D. S., R. J. Sayler, Y. G. Hong, M. H. Nam and Y. Yang. 2008. A method for inoculation and evaluation of rice sheath blight disease. Plant Dis. 92:25-29.

Endo, S. 1933. Effect of sunlight on the infection of the rice plant by *Hypochnus sasakii* Shirai. Ibid. 8: 75-48.

Yoshimura, S. 1955. On the effect of the shading upon the susceptibility of the rice plant to the sheath blight *Hypochnus sasakii* Shirai. Ibid.16: 113.

Burpee, L. L. and L. G. Goulty. 1984. Evaluations of fungicides for control of pink and gray snow mold on creeping bentgrass. In: R. W. Sheard (Ed.), Turfgrass Research Annual Report, Vol. 38. University of Guelph, Ontario, pp. 6-7.

Yoshie, Y., I. Katsushige, U. Yoshihisa, O. Atsuko, T. Kazutoh and N. Hiroshin. 1993. Isolation, structures, and antifungal activities of new Aureobasidins. J. Antibiotics. 46: 1347-1354.

Han, B. H., Y. N. Han, K. A. Han, M. H. Park and E. O. Lee. 1983. Studies on the anti-inflammatory activity of *Aralia continentalis* characterization of continentakic acid and its anti-inflammatory activity. Arch. Pharm. Res. 6: 17-23.

Han, W. S. 2004. Isolation of antimicrobial compounds from *Salvia miltiorrhiza* Bunge. Korea J. Med. Crop Sci., 12(3): 179-182.

Oh, T. S. and C. H. Kim. 2013. Composition for controlling of rice blast containing Aralia continentalis extract and a method of controlling rice blast using the composition, Korean Patent, Korea Intellectual Property Office 10-1245582.