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Research article

Fungicidal activity of *Acorus calamus* L. extracts against plant pathogenic fungi

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Abstract

The efficacy was tested *in vitro* of *Acorus calamus* L. crude ethanol extract against eight plant pathogenic fungi: *Alternaria brassicicola*, *Colletotrichum capsici*, *Bipolaris oryzae*, *Lasiodiplodia theobromae*, *Phytophthora palmivora*, *Pyricularia oryzae*, *Rhizoctonia solani* and *Sclerotium rolfsii*. The results showed that the extract completely inhibited the radial growth of all tested fungi except *A. brassicicola* at a concentration of 10 g/L as well as significantly inhibiting the rice pathogens, *B. oryzae* and *R. solani* by 78.52% and 74.19%, respectively, at a concentration of 1 g/L. The extract was further analyzed using chromatographic techniques, and the solvent extracts were investigated for their antifungal effect on *B. oryzae* and *R. solani*. The active solvent extracts against both pathogens in petroleum ether and the chloroform extracts led to the isolation of two antifungal compounds, β-asarone and galangin, which exhibited antifungal activity against *B. oryzae* and *R. solani* with minimal inhibitory concentration values of 0.5 g/L and 0.125–0.25 g/L, respectively. Under greenhouse conditions, application of crude ethanol extract 1 d before *B. oryzae* inoculation at 5 g/L significantly reduced rice brown spot incidence by 47% compared to the negative control.

Introduction

Plant disease management strategies have been developed for combating economically destructive pathogens; these include eco-friendly approaches such as the use of biological control agents or plant-derived fungicides (Yoon et al., 2011). The search for potential plant-derived fungicides has received increased attention due to the disadvantages of fungicide use which have been reported, and other strategies have been found to be not as successful for plant disease protection from aggressive plant pathogen infections (Gahukar, 2012). Natural products from Thai medicinal plants are well known as promising sources for developing alternatives to conventional fungicides since some of their metabolites have been proved to possess antimicrobial activities against either plant or human pathogens (Divya et al., 2011; Plodpai et al., 2013a, 2013b; Shetty and Shruthi, 2015). These effects were influenced by their chemical composition via substances acting directly against plant pathogens or indirectly involved in other mechanisms (Ujvary, 2002). The eight phytopathogenic fungi used in the current study are main pathogens affecting economic crops including rice, vegetables and fruits in Thailand as well as in many other countries worldwide (Plodpai et al., 2013a, 2013b; Dethoup et al., 2015; Jaihan et al., 2016). Farmers usually apply fungicides to control these pathogens; however, repeated applications of fungicides have resulted in the development of pathogen resistance (Thind, 2012). Hence, there is an urgent need to find an effective, safe and eco-friendly strategy to replace synthetic fungicides.

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Acorus calamus L. (common name, sweet flag), belonging to the Acoraceae family, is found in Asia and Eastern Europe (Singh et al., 2010). Its metabolites have been reported to display various biological activities (Kumar et al., 2015b; Shetty and Shruthi, 2015). Due to its various biological activities, investigations of metabolites from its leaves and rhizomes were conducted and reported (Dong et al., 2010; Liu et al., 2013; Suman et al., 2014; Jaiswal et al., 2015). β-Asarone, α-asarone, (*E*)-methylisoeugenol, (-)-surinamensinol A, isoeugenol, (+)-shyobunone, 3,4-dimethoxycinnamic acid, β-linalool, δ-cadinene and *trans*-cadinol are some major constituents of *A. calamus* L. (Liu et al., 2013; Jaiswal et al., 2015), among which β-asarone was a key antifungal constituent in *A. calamus* L. extracts (Dissanayake et al., 2015). Crude *A. calamus* L. extracts have been reported to display broad antimicrobial activities toward a variety of plant and human pathogens (Bhuvaneswari and Balasundaram, 2006, 2009; Kumar et al., 2015a, 2015b). However, evaluation of the antagonistic effects toward all the plant pathogenic fungi (except *R. solani*) used in this study is still limited.

The objectives of this study were to investigate: 1) the antifungal effects of *A. calamus* L. extract on eight plant pathogenic fungi *in vitro*, 2) the antifungal substances responsible for the antifungal activity against the tested plant pathogens from the active fractions and 3) the fungicidal activity of *A. calamus* L. extract to control brown spot and sheath blight of rice seedlings under greenhouse conditions.

Materials and Methods

Plant material

Rhizomes of *A. calamus* L. were used in this study in accordance with their use in traditional medicine. *Acorus calamus* L. rhizomes were purchased from the medicinal plant market, 'Saphanmai', in Bangkok, Thailand. After botanical identification of the species, a voucher specimen was deposited at the Natural Products for Plant Protection Division, Kasetsart University, Bangkok, Thailand. The rhizomes were washed with tap water, air dried in the shade, then ground to a fine powder using a high power blender and kept at 4°C until used.

Plant extraction

The plant powder (200 g) was extracted twice using maceration with 70% ethanol (2 L) for 1 wk at room temperature. The aqueous ethanol extracts were combined and filtered through three layers of sterile cheesecloth and then concentrated under reduced pressure to obtain a crude ethanol extract. The extract (10 g) was further successively extracted using organic solvents to obtain petroleum ether (317.7 mg), chloroform (1,245 mg), diethyl ether (294.6 mg), ethyl acetate (135.2 mg), acetone (98.5 mg), and methanol (85.3 mg) extracts. All extracts were maintained in vials and kept at 4 °C until used.

Fungal pathogens

The phytopathogenic fungi assayed in this study (Table 1) were isolated from infected plants and their pathogenicity was confirmed following Koch's postulation method (Schneider and Alan, 2010). The pathogens were identified based on morphological characteristics and molecular techniques using internal transcribed spacer primers; their gene sequences were deposited in GenBank with accession numbers, as shown in Table 1. The proven strains were deposited at the Laboratory of Natural Products for Plant Protection, Department of Plant Pathology, Kasetsart University, Bangkok, Thailand.

In vitro radial growth inhibition test

The antifungal activity of crude ethanol extract was evaluated on eight plant pathogenic fungi *in vitro* using the dilution plate method as described by Dethoup et al. (2015). Briefly, the crude extract was dissolved in dimethyl sulfoxide (DMSO) and serially diluted in sterile water and then mixed with warm potato dextrose agar (PDA) to achieve final concentrations of 10 g/L, 1 g/L and 0.1 g/L in separate Petri dishes. A mycelial plug (5 mm in diam) from colonies aged 7 d of each pathogen culture was placed on the center of a Petri dish containing PDA-amended crude extract of each concentration and incubated for 3 d for *L. theobromae*, *R. solani* and *S. rolfsii*, and 10 d for the remaining species at room temperature. A sample of 1% DMSO was used as the negative control. Five replications were made of each treatment. The radial growth inhibition (RGI) was calculated according to the formula: %RGI = (R1–R2)/A × 100, where R1 = the diameter of the radial growth of each pathogen of the negative control and R2 = the diameter of the radial growth of each pathogen treated with extract at the three concentrations. Since the *A. calamus* L. crude extract revealed potent inhibitory effects on the radial growth of *R.*

Table 1 Effects of *Acorus calamus* L. crude ethanol extract against eight plant pathogenic fungi *in vitro*.

Plant pathogen	Accession number	% Radial growth inhibition		
		10 g/L	1 g/L	0.1 g/L
Alternaria brassicicola (Schwein.) Wiltshire	MH749465	91.67 ± 0.59^{b}	38.15 ± 2.12^{h}	9.62 ± 1.28^{k}
Colletotrichum capsici (Syd. & P. Syd.) E.J. Butler & Bisby	MH749466	100 ± 0^{a}	43.70 ± 1.29^{g}	5.56 ± 0^{l}
Bipolaris oryzae (Breda de Haan) Shoemaker	MG914430	100 ± 0^{a}	78.52 ± 1.54^{c}	27.78 ± 5.56^{j}
Lasiodiplodia theobromae (Pat.) Griffon & Maubl.	MH724312	100 ± 0^{a}	69.63 ± 0.59^{e}	0 ± 0^{n}
Phytophthora palmivora Butler (Butler)	MH749467	100 ± 0^{a}	3.70 ± 0.98^{m}	0 ± 0^{n}
Pyricularia oryzae Cavara	MH749468	100 ± 0^{a}	35.56 ± 0.83^{i}	5.19 ± 0.64^{lm}
Rhizoctonia solani J.G. Kühn	MH749469	100 ± 0^{a}	74.19 ± 0.92^{d}	39.63 ± 2.31^{h}
Sclerotium rolfsii Sacc.	MH749470	100 ± 0^{a}	62.96 ± 8.54^{f}	0 ± 0^{n}

Values (mean ± SD) followed by the same lowercase superscript do not significantly difference at $p < 0.05$.

solani and *B. oryzae*, the six solvent extracts obtained from successive extractions of *A. calamus* L. crude ethanol extract were evaluated for their potential radial growth inhibition on both fungi by the dilution plate method at 10 g/L, 1 g/L and 0.1 g/L as mentioned above. All experiments were repeated three times.

Isolation and elucidation of pure compounds

Based on the *in vitro* antifungal activity tested, petrol and chloroform extracts revealed the strongest activity towards the tested pathogens, and thus these two active extracts were selected for further chemical analysis. Both extracts were combined (1.56 g) and applied on a chromatography column (3 × 50 cm, Kiesel gel 60, 70–230 mesh, Merck, Germany) and eluted stepwise with petrol-$CHCl_3$ at ratios of 1:4, 2:3 and 4:1, respectively, wherein 50 mL fractions were collected. Fractions 5–7 (572.9 mg), eluted with petrol-$CHCl_3$ 2:3, were combined and crystallized in a mixture of petrol and $CHCl_3$ to give 92.2 mg of galangin. The mother liquor, after evaporation of the solvents, produced 145.6 mg of a brown oil of β-asarone. Both compounds were characterized using analysis of their 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC and HMBC) nuclear magnetic resonance (NMR) data as well as comparisons of their NMR data with those in the literature.

Minimal inhibitory concentration bioassay

The lowest concentrations of pure compounds and the crude extract that completely inhibited fungal mycelial growth were determined *in vitro* in 96-microwell plates. Each sample was dissolved in DMSO, and a two-fold serial dilution in potato dextrose broth (PDB) medium to achieve concentrations in the range 0.0625–1 g/L with a final volume of 100 µL in each well. A mycelial plug (3 mm in diam) from colonies aged 7 d of *R. solani* and *B. oryzae* cultures was placed separately in each well which contained the prepared mixture of PDB and one of the tested substances of a certain concentration. Again, 1% DMSO was used as the negative control. The plates were incubated for 48 hr at room temperature. Five replications were made of each treatment and repeated more than twice. The minimal inhibitory concentration (MIC) value was assessed by observation of the lowest concentration that completely inhibited the radial growth of the tested pathogens.

Suppression brown spot disease on rice seedlings in greenhouse tests

Rice seeds (cv. Hom Mali 105) were surface disinfected with 1% (volume per volume, v/v) sodium hypochlorite for 5 min, rinsed three times with sterile water and then soaked in sterile water for 24 hr for imbibition. After this, they were sown in tray nurseries and left for 7 d. At which tome five seedlings of the same height and vigor were transplanted to a plastic pot (15 cm diam, 11 cm in height) containing sterile clay soil (500 g), placed in a greenhouse at 28 ± 3°C for 5 wk and watered daily to maintain a water level 1 cm above the soil surface throughout the experiment. The crude ethanol extract was dissolved in DMSO and diluted in sterile water to obtain the final concentrations of 5 g/L, 1 g/L and 0.5 g/L. In the preventive test, five rice plants aged 6 wk in a pot were sprayed with 30 mL of each concentration of the extract on both sides of the leaves at 24 hr before pathogen inoculation. In the curative test, 30 mL of the crude extract of *A. calamus* L. was sprayed on the five rice plants aged 6 wk in a pot at 24 hr after pathogen inoculation. In both the preventive and curative tests, the rice seedlings were inoculated with 30 mL of a spore suspension of *B. oryzae* (1×10^6 spores/mL) containing 0.02% Tween-20 (v/v) per pot and incubated at 28 ± 3°C and 95–100% humidity for 24 hr. Rice seedlings treated with 1% DMSO and 0.02% Tween-20 without crude extract of *A. calamus* L. were used as the negative control. Disease incidence was recorded 1 wk after pathogen inoculation wherein three leaves were collected from the middle part of each plant (in total, 75 rice leaves from 25 plants per treatment). The disease severity was recorded according to the scoring scale presented by International Rice Research Institute (2013) in which 0 = no infection observed, 1 = lesions were limited to less than 1% of the leaf area, 2 = lesions covered 1–3%, 3 = lesions covered 4–5%, 4 = lesions covered 6–10%, 5 = lesions covered 11–15%, 6 = lesions covered 16–25%, 7 = lesions covered 26–50%, 8 = lesions covered 51–75% and 9 = lesions covered >75% of the leaf area.

Each treatment consisted of five pots with five rice seedlings in each pot, and the assay was repeated more than twice. The pots of treated plants were arranged in greenhouse with a completely randomized design.

Suppression of sheath blight disease on rice seedling in greenhouse tests

The fungicidal activity evaluation of *A. calamus* L. crude extract against sheath blight at concentrations of 5 g/L, 1 g/L and 0.5 g/L was conducted according to Rosas et al. (2016) with a minor modification. In the preventive test, five seedlings aged 6 wk in a pot were sprayed with 30 mL of crude ethanol extract of a concentration per pot at the basal part up to 15 cm from soil level before pathogen inoculation. In the curative test, 30 mL of the *A. calamus* L. crude extract was sprayed on five rice seedlings aged 6 wk at 24 h after pathogen inoculation. In both the preventive and curative tests, each rice seedling was inoculated using an *R. solani* disc (0.5 cm in diam) from a culture aged 7 d attached at the basal part of the stem at 3 cm above the soil surface and sealed with plastic film to prevent drying. Rice seedlings treated with 1% DMSO without crude extract of *A. calamus* L. were used as the negative control. Disease incidence was recorded according to the 0–9 disease incidence scale of Rosas et al. (2016) in which 0 = no infection, 1 = lesions were limited to less than 20% of plant height, 3 = lesions covered 21–30% of plant height, 5 = lesions covered 31–45% of plant height, 7 = lesions covered 46–65% of plant height and 9 = lesions covered >65% of plant height.

Each treatment consisted of five pots with five rice seedlings in each pot, and the assay was repeated more than twice. The pots of treated plants were arranged in greenhouse using a completely randomized design.

Statistical analysis

Due to the non-significant differences between the repeated experiments of each treatment at $p < 0.05$ after analysis by variance and means were compared using Duncan's multiple range test ($p < 0.05$), using the statistical program SPSS version 19 (IBM Corporation; NY, USA). Thus, the data obtained from the repeated experiments were pooled and submitted for analysis as mention above.

Results

Antifungal activity of A. calamus L. *crude ethanol extract against eight plant pathogenic fungi in vitro*

Acorus calamus L. crude ethanol extract showed complete radial growth inhibition on all tested plant pathogenic fungi, except *A. brassicicola*, at the highest dose tested and exhibited the best antifungal effect against *B. oryzae* causing 100% and 78.52% radial growth inhibition at concentrations of 10 and 1 g/L, respectively. It also displayed strong radial growth inhibition of *R. solani, L. theobromae* and *S. rolfsii*, causing over 60% inhibition at a concentration of 1 g/L (Table 1).

In vitro antifungal activity of different solvent extracts of A. calamus L.

The results from the antifungal activity evaluation of the six different solvent extracts against *B. oryzae* and *R. solani* revealed that only the petroleum ether and chloroform extracts exhibited effects on both plant pathogenic fungi (Table 2); the rest of the extracts did not show activity against either tested pathogens even at the highest dose tested (data not shown).

Antifungal compounds isolated from A. calamus L. *active solvent extractions*

β-Asarone and galangin were isolated and purified using chromatographic techniques and characterized using spectroscopic methods. β-Asarone (Siergiejczyk et al., 2000) was isolated as a pale brown oil and the analysis results were: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H:1.85 (dd, J_H = 6.8, 1.6), 3.81 (3H, s), 3.84 (3H, s), 3.90 (3H, s), 5.77 (dd, J_H = 11.2, 6.8, H-7'), 6.50 (dd, J_H = 11.2, 2.0, H-8'), 6.55 (s), 6.86 (s). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C:14.7 (C-9), 56.3 (C-4), 56.4 (C-2), 56.6 (C-6), 97.6 (C-3), 114.2 (C-6), 124.2 (C-7), 124.8 (C-8), 142.5 (C-1), 146.5 (C-4), 148.8 (C-2), 151.6 (C-5). Galangin (Wawer and Zielinska, 2001) was isolated as a pale yellow powder and the analysis results were ^{1}H-NMR ($CDCl_3$, 400 MHz) δ [ppm] 6.27 (d, J_H = 2.0), 6.47 (d, J_H = 2.0), 7.54 (m, H-3', H-5'), 7.55 (2H, m, H-4'), 8.14 (2H, dd, J_H = 7.2, 1.6, H-3'), 12.36 (s). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ [ppm]: 93.6 (C-8), 98.4 (C-6), 103.2 (C-4a), 127.6 (C-2', 6'), 128.6 (C-3', 5'), 130.0 (C-4'), 131.0 (C-1'), 137.1 (C-3), 145.8 (C-2), 156.5 (C-8a), 160.8 (C-5), 164.3 (C-7), 176.3 (C-4).

Minimal inhibitory concentration of A. calamus L. *extract and antifungal substances*

The MIC values of *A. calamus* L. crude ethanol extract on *B. oryzae* and *R. solani* were 3.5 and 0.7 g/L, respectively. The MIC values of β-asarone and galangin on *B. oryzae* were both 0.5 g/L; however, β-asarone displayed higher antifungal activity (MIC of 0.125 g/L) than galangin (MIC of 0.25 g/L) on *R. solani*.

Effects of A. calamus L. extract on brown spot and sheath blight disease suppression under greenhouse conditions

The fungicidal effects of *A. calamus* L. crude ethanol extract on brown spot disease of rice under greenhouse conditions are shown in Table 3 and Fig. 1. Treatment with the extract at the highest dose tested before pathogen inoculation resulted in a significant reduction in the incidence of brown spots by 47%, compared with the negative control, as well reducing the extension of the brown spot after pathogen inoculation. The extract did not significantly suppress sheath blight, compared with the negative control for all tested concentrations (data not shown). Phytotoxicity of the extract on rice seedlings was observed when treated at the high concentration of 10 g/L.

Discussion

The ethanol crude extract of *A. calamus* L. revealed a wide spectrum of antifungal activity against various classes of plant pathogenic fungi except those belonging to the class Oomycetes, since the current result indicated that *Ph. palmivora* was less sensitive to the extract. *A. calamus* L. is normally available and commonly used in a

Table 2 Effects of solvent extracts of *Acorus calamus* L. on *Bipolaris oryzae* and *Rhizoctonia solani*.

Solvent extract	Concentration (g/L)	% Radial growth inhibition	
		Bipolaris oryzae	*Rhizoctonia solani*
Petroleum ether extract	10	100.00^{a}	100.00^{a}
	1	74.72 ± 2.21^{c}	100.00^{a}
	0.1	0.00^{f}	46.67 ± 3.27^{e}
Chloroform extract	10	100.00^{a}	93.11 ± 7.39^{b}
	1	63.89 ± 1.11^{d}	0.00^{f}
	0.1	0.00^{f}	0.00^{f}

Values (mean ± SD) followed by the same lowercase superscript do not significantly differ at $p < 0.05$, based on Duncan's multiple range test of one-way analysis of variance.

Table 3 Effects of *Acorus calamus* L. crude ethanol extract application on disease severity of rice brown spot.

Treatment	Protective test		Curative test	
	Brown spot score (1–9 scale)	% Disease reduction	Brown spot score (1–9 scale)	% Disease reduction
A. calamus L. extract (5 g/L)	0.95[a]	47.01 ± 3.27[a]	4.69[b]	33.29 ± 2.65[b]
A. calamus L. extract (1 g/L)	3.60[b]	33.44 ± 2.35[b]	5.25[bc]	26.57 ± 5.11[c]
A. calamus L. extract (0.5 g/L)	4.11[b]	28.87 ± 4.69[c]	6.50[cd]	14.91 ± 6.21[d]
Water (control)	7.24[d]	–	7.31[d]	–

Values (mean ± SD) within columns of brown spot score and % disease reduction followed by the same lowercase superscript are not significantly different at $p < 0.05$ using Duncan's multiple range test of one-way analysis of variance.

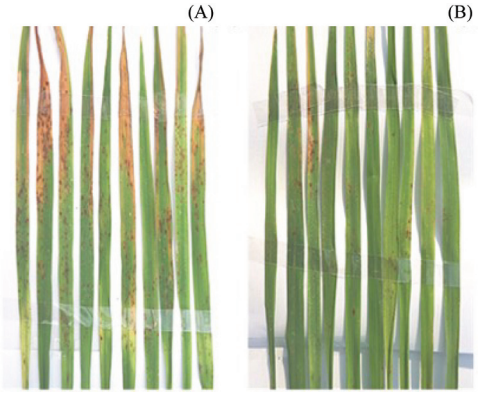

Fig. 1 Effect of *Acorus calamus* L. crude ethanol extract against rice brown spot when applied at 1 d before *B. oryzae* inoculation: (A) 0 g/L (negative control); (B) 0.5 g/L

traditional medicine in Asia, and the antimicrobial activities have been previously investigated of *A. calamus* L. extracts on various human pathogens as well as some plant pathogens. For example, Dissanayake et al. (2015) reported the complete growth inhibition of *A. calamus* L. extract against *A. solani*, *B. cinerea* and *C. gloesporioides* at a concentration of 5 g/L, but the same author reported the extract displayed weak antifungal activities on *R. solani*, *C. musea,* and *F. oxysporum* showing 84–96% inhibition at a concentration of 20% (Dissanayake and Jayasinghe, 2013). This contrasts with the current results, since in the current study the *A. calamus* L. crude ethanol extract completely inhibited growth of *R. solani* at a concentration of 10 g/L, although the extract failed to control sheath blight under greenhouse conditions. These conflicting results may have resulted from the different amounts of active constituents in *A. calamus* L. grown in different locations and climates. However, the environmental effects may have resulted in the fungicidal activity of *A. calamus* L. crude ethanol extract not suppressing rice sheath blight under the greenhouse conditions. The results from previous studies and the current study indicated that *A. calamus* L. extracts possess wide antifungal activity against plant pathogenic fungi at various concentrations *in vitro*.

In accord with the current results, Lee et al. (2007) reported that the hexane-fraction of *A. gramineus* extract at 2 g/L displayed a strong antifungal effect on *R. solani* growth. In addition, several other studies found that the essential oils isolated from *A. calamus* L. rhizomes exhibited broad biological activities on plant pests (Sagar and Rana, 2012; Liu et al., 2013), which indicated that the bioactive compounds in *A. calamus* L. extracts responsible for the antifungal activity have low polarity and thus dissolve well in nonpolar organic solvents. Researchers have previously reported the isolation of β-asarone from *A. calamus* L. extracts, which was a potent antifungal substance against plant pathogens (Shenvi et al., 2011; Dissanayake et al., 2015). These results confirmed that antifungal substances produced by *A. calamus* L. were nonpolar compounds.

However, β-asarone and galangin isolated from *A. calamus* L. rhizomes in the current study, had higher antifungal activity on *B. oryzae* and *R. solani* than the essential oils isolated from *Lippia geminate* and *Cymbopogon jwarancusa* since the half maximal inhibitory concentrations of their essential oils on both pathogens were in the range 336.74–481.47 µg/mL (Bhuyan et al., 2010). The antifungal activities of bioactive compounds isolated from the other *Acorus* species have been studied and β-asarone isolated from *A. gramineur* displayed antifungal activity against *C. cucumerium*, *C. orbiculare*, *M. grisea* and *P. ultimum* with MIC values in the range 0.5–30 µg/mL (Lee et al., 2004). This confirmed that β-asarone was responsible for the antifungal effect on various species of plant pathogens. However, the current study provided novel information about the other antifungal substance besides β-asarone present in the *A. calamus* L. rhizome extract.

Since brown spot and sheath blight diseases are considered as major diseases of rice worldwide, many attempts have been made to find botanical fungicides against these diseases. For example, Khoa et al. (2011) reported that treatment with 20 g/L *C. odorata* leaf extract could reduce the lesion length of rice sheath blight up to 48% and treatment of rice seed with 50 g/L of this extract reduced brown spot symptoms by 57%. Nguefack et al. (2013) reported that rice brown spot severity was reduced by 36–42% when treated with a combination of essential oils, 20 g/L extracts of *Callistemon citrinus* L. and *Cymbopogan citratus* (DC) Stapf, as a seed dressing and plant spray. However, when the current results are compared with the results of these previous studies, it seems that *A. calamus* L. rhizome extract has higher antifungal activity against brown rice spot, since it caused a great reduction in disease incidence at lower concentrations both in the laboratory and under greenhouse conditions.

In conclusion, *A. calamus* L. extract had significant antifungal activity both *in vitro* and under greenhouse conditions against *B. oryzae*, a causal agent of rice disease. β-Azarone and galangin present in low polar solvent extracts were the major antifungal compounds active against the tested plant pathogenic fungi. The current findings suggested that the *A. calamus* L. extract is a promising candidate as a botanical fungicide to control brown spot of rice caused by *B. oryzae*.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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