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Gallic acid isolated from *Sedum lineare* and its control efficacy on rice bacterial leaf streak

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Abstract: A compound, which could significantly inhibit the growth of *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) *in vitro*, was isolated from *Sedum lineare* by liquid-liquid extraction, silica gel column chromatography and gel column chromatography. Based on the spectral data, including ^{1}H and ^{13}C NMR data, this compound was identified as 3,4,5-Trihydroxybenzoic acid, namely gallic acid(GA). The results of the test, on determining the antibiotic spectrum of GA at concentration 30 mg · mL^{-1}, indicated that GA could most strongly inhibit the growth of *X. campestris* pv. *pruni*, *X. oryzae* pv. *oryzicola*, *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *citri*, with inhibition zones width of 25.33, 24.00, 22.33 and 20.67 mm, respectively, and had fair anti-bacteria activity against *Ralstonia Solanacearum*, *Pseudomonas syringae* pv. *glycinea*, *P. syringae* pv. *tomato* and *Pectobacterium carotovora* subsp. *carotovora*, with inhibition zones width of 18.00, 12.33, 11.00 and 8.68 mm, respectively. Meanwhile, GA could weakly inhibit the growth of eleven pathogenic fungi, including *Phytophthora nicotianae*, *Penicillium digitatum*, *Streptobotrys streptothrix*, *Pythium aphanidermatum*, *Pestalotiopsis mangiferae*, *Curvularia lunata*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *niverum*, *F. oxysporum* f. sp. *nicotianae*, *Botrytis cinerea* and *Sclerotium rolfsii*, with inhibition ratio of 56.34%, 54.73%, 38.00%, 29.86, 28.17%, 20.00%, 19.02%, 16.71%, 10.59%, 4.58% and 1.96%, respectively. Growth inhibition exceeding 50% by GA was only observed in *Phytophthora nicotianae* and *Penicillium digitatum*. The growth of *Botrytis cinerea* and *Sclerotium rolfsii* was slightly inhibited by GA. In potted experiment, gallic acid at 200, 300 and 400 mg · L^{-1} provided 63.55%, 71.38% and 77.39% control efficacy to rice bacterial leaf streak, respectively. At concentration of 300 mg · mL^{-1}, GA could control rice bacterial leaf streak, with control efficacy of 64.62% in field. The study reveals that the gallic acid has the potential to be further developed as a bactericide to control rice bacterial leaf streak.

Key words: anti-bacterial substance, extraction and purification, *Sedum lineare*, gallic acid, rice bacterial leaf streak, control efficacy

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抑制水稻细菌性条斑病菌的没食子酸分离及其对水稻细菌性条斑病的防治作用

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摘 要: 通过液—液萃取、硅胶和凝胶柱层析法,从佛甲草(*Sedum lineare*)分离出一种可以抑制水稻细菌性条斑病菌(*Xanthomonas oryzae* pv. *oryzicola*, *Xoc*)生长的单体化合物,经质谱分析,确定该化合物为没食子酸(gallic acid, GA)。在 30 mg·mL^{-1}浓度下,GA 能抑制一些植物病原细菌如桃细菌性穿孔病菌(*X. campestris* pv. *pruni*)、水稻细菌性条斑病菌(*X. oryzae* pv. *oryzicola*)、水稻白叶枯病菌(*X. oryzae* pv. *oryzae*)、柑橘溃疡病菌(*X. axonopodis* pv. *citri*)、大豆细菌性斑点病菌(*Pseudomonas syringae* pv. *glycinea*)、番茄细菌性斑点病菌(*P. syringae* pv. *tomato*)和胡萝卜软腐果胶杆菌(*Pectobacterium carotovora* subsp. *carotovora*)的生长;GA 还对 11 种植物病原真菌如烟草疫霉(*Phytophthora nicotianae*)、指状青霉(*Penicillium digitatum*)、滇刺枣褐腐病菌(*Streptobotrys streptothrix*)、瓜果腐霉(*Pythium aphanidermatum*)、芒果拟盘多毛孢(*Pestalotiopsis mangiferae*)、新月弯孢霉(*Curvularia lunata*)、立枯丝核菌(*Rhizoctonia solani*)、(*Fusarium oxysporum* f. sp. *niverum*)、西瓜专化型尖孢镰刀菌(*F. oxysporum* f. sp. *nicotianae*)、番茄灰霉病菌(*Botrytis cinerea*)和齐整小核菌(*Sclerotium rolfsii*)的生长具有一定的抑制作用。在 300 mg·mL^{-1}浓度下,GA 对水稻细菌性条斑病的田间防治效果达到 64.62%。该研究结果表明没食子酸具有开发成为一种防治水稻细菌性条斑病的杀菌剂的潜力。

关键词: 抑菌物质, 提取和纯化, 佛甲草, 没食子酸, 水稻细菌性条斑病, 防治效果

Rice bacterial leaf streak, caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), is one of the major rice diseases in the rice planting areas of tropical and subtropical zones. In China, the disease was firstly spotted in Pearl River Delta in China, and prevailed in Guangdong, Guangxi, Hainan, Sichuan and Zhejiang in 1950s -1960s (Fang et al, 1957). This disease could result in 15% - 20% loss of production, even 40% - 60%, and is the quarantined disease in China (Raymundo & Briones, 1995; Nino-Liu et al, 2006). In the past few years, because breeding for resistance did not resulted in useful varieties yet, the disease brought great loss to the safe production of rice (Levy et al, 1991; He et al, 2012). Application of bactericide is the major strategy for control of this disease but, at present, few bactericides, such as Zn-thiazole and thiodiazole-copper etc., are registered in China (Wei et al, 2007; Zhu et al, 2010). It is desirable to develop a new bactericide to manage this disease. *Sedum lineare* is a perennial herb in the genus *Sedum*, family Crassulaceae. The plant lives in the condition of drought and coldness, and is easy to grow. Therefore, it is mainly used for roof greening (Feng et al, 2010; Liu et al, 2012; Lu et al, 2015). It has been found some medical applications of the extracts of *Sedum lineare* (Niu et al, 2014; Rouhier & Jacquot, 2005; Zhou et al, 2013). In the early works on screening of plants for antibacterial activity against *Xoc*, we found that the methanol extracts of *S. lineare* could significantly inhibit the growth of *Xoc in vitro*.

At present, there are no report on the using antimicrobial substances of *S. lineare* to control plant diseases. The objective of this study was to isolate and identify the compound responsible for the antibacterial activity against *Xoc* from *S. lineare*, and to evaluate the effects of selected compounds for control of rice bacterial leaf streak.

1 Materials and Methods

1.1 Pathogen and culture conditions

Xoc XD1109 was used in the screening of prospective plant constituents and greenhouse experiments. Other strains, listed in tables 1 and 2, were used as determining the antibiotic spectrum of gallic acid. All strain used in this study were provided by the Plant Pathology Research Institute, College of Agriculture, Guangxi University, China.

Strains of pathogenic bacterium were cultured initially on nutrient agar (NA: beef extract 3 g, peptone 8 g, agar 18 g, distilled water 1 000 mL) for 24–48 h and then transferred into nutrient agar for suspension cultivation (28 ℃, 130 r · min^{-1}) for 24 h. Strains of pathogenic fungi were cultured on potato sucrose agar, (PSA: potato 200 g, sucrose 20 g, agar 15 g, distilled water 1 000 mL) at 28 ℃ for 7–15 d.

1.2 Preparation of methanol extract of *S. lineare*

The samples of *S. lineare*, collected from Guangxi University, Nanning, Guangxi, China in 2012, were dried in an oven under the temperature of 50 ℃, then grounded into powder using 40-mesh screen (the diameter of the holes was 0.37 mm). The powder (200 g) was soaked in methanol of volume eight times of the powder at room temperature for 100 h. Methanol solution was evaporated under reduced pressure in water of permanent 55 – 65 ℃ with a rotary evaporator to obtain crude extract.

1.3 Isolation of anti-bacterial substance

According to the different polarities of the components, solvent extraction method described by Yao & Wen(2011) was used to isolate anti-bacterial substance in methanol extract of *S. lineare*. The methanol extract of *S. lineare* was suspended in water (1 ∶ 2 by volume) and extracted four times with petroleum ether. The petroleum ether extract was evaporated to remove the solvent under reduced pressure in water of permanent 55–65 ℃ with a rotary evaporator, and the remaining aqueous layer was successively extracted with chloroform, ethyl acetate, and n-butanol. Each extract was concentrated to dryness by a rotary evaporator and tested its antibacterial activity against *Xoc in vitro*.

1.4 Purification of anti-bacterial substance

The crude extract of anti-bacterial substance obtained from solvent extract was subjected to silica gel column GF254 (Qingdao Haiyang Chemical Industry Factory) chromatography. A total 20 g of dried crude extract was eluted with chloroform and methanol. The proportions of their volumes were 40 ∶ 1, 20 ∶ 1, 10 ∶ 1, 5 ∶ 1, 2 ∶ 1, 1 ∶ 1 by volume, and finally with methanol. Eluant was collected into fractions of 400 mL, and then was evaporated under reduced pressure in water of permanent 55–65 ℃ with a rotary evaporator.

Thin layer chromatography (TLC) was used to determine their purity of fractions. The fractions, showing the same TLC profiles, were combined and tested their antibacterial activity *in vitro* by the agar well diffusion method.

The fractions, which had antibacterial activity against *Xoc*, were dissolved in methanol and filtered by a membrane with holes (0.22 μm in diameter), and then purified by Sephadex LH-20 (Pharmacia, USA) column (100 cm × 0.8 cm). 0.55 g of sample (20 mg · mL^{-1}) was loaded into the column and eluted with methanol. Eluant was collected into fractions of 5 mL. The antibacterial activities of all fractions was tested *in vitro* by the agar well diffusion method.

1.5 Structure identification

Nuclear magnetic resonance (NMR) method (Ibargoitia et al, 2014; Kwang-Hyun et al, 2014) was used to determine the structure of the monomeric compound isolated from *S. lineare*, which had antibacterial activity against *Xoc*. Mass spectrum was recorded by electrospray ionization mass spectrometry. NMR spectra were recorded on a superconducting flourier transform NMR spectrometer (INOAVA, 600 MHz; Varian, America) at 600 MHz for ^{1}H and at 150 MHz for ^{13}C, with TMS as an internal standard.

1.6 Determination of *in vitro* anti-bacterial activity

Antibacterial activity of extracts from *S. lineare* was tested by the agar well diffusion method described by Yang et al (2010). The nutrient agar medium containing *Xoc* (10^8 cfu · mL^{-1}) was poured into petri plates and allowed to set. A 7 mm cork borer was used to bore holes on the medium. About 70 μL of the extract solution was introduced into the well. The extracts of *S. lineare* were dissolved with 10% methanol. The wells loaded with the same concentration of solvent served as controls and 0.1 mg · mL^{-1} streptomycin as positive control. Each treatment consisted of three replicates. Inhibitory diameter of the well in each treatment was measured at 48 h after incubation at 28 ℃.

1.7 Determination of *in vitro* antifungal activity

Antifungal activity of gallic acid (GA) was tested by a mycelia radial growth inhibition test (Yuan et al, 2010). The test strains were cultured on potato sucrose agar at 28 ℃ for 7 d. A mycelium disc (5 mm in diameter) of the test strains, which was cut with cork borer from the periphery of young cultures, was placed in the center of potato sucrose agar medium plates containing GA at concentration 30 mg · mL^{-1}. GA was dissolved with methanol. Control plates were treated with methanol at a concentration of 0.1% (vol · vol^{-1}). Each treatment consisted of three replicates. The plates were incubated at 28 ℃ for 7−15 d. Growth inhibition of the treatment were calculated using the following formula:

Growth inhibition = $100 \times (C-T) / C$, where C is the colony diameter of the control and T is the colony diameter of the GA-treated plate.

1.8 Determination of disease control efficacy

Potted experiments: The rice cultivar "Boyou 680" was cultivated in a 20-cm-diameter pots (10 plants per pot) in a greenhouse at (30 ± 5) ℃. The suspension (10^8 cfu · mL^{-1}) of *Xoc* was sprayed on rice leaf at tillering stage (10 mL per plant). Gallic acid at the concentrations of 200, 300 and 400 mg · L^{-1} was sprayed on rice leaves (10 mL per plant) 24 h after *Xoc* inoculation. Meanwhile, water (control) and 20% thiodiazole-copper suspension concentrate at the concentration of 570 mg · L^{-1} were sprayed on rice leaf (10 mL per plant) 24 h after *Xoc* inoculation. Each treatment consisted of four replicates and each replicates contained 30 plants. The disease severity was investigated 15 d after pathogen inoculation.

The disease severity was divided into six ratings (Luo et al, 2011): 0 = no symptom, 1 = only a small spot of lesion and less than 1% the leaf area infected, 3 = scattered short steaks of lesions and 1%−5% the leaf area infected, 5 = plenty of lesions on the leaf and 6%−25% leaf area infected, 7 = 26%−50% leaf area infected and 9 = more than 51% leaf area infected. The disease index(DI) and control efficacy were calculated using the following formulae:

DI = ∑ (the value of each level × the number of leaves that are at this level of infection)/(the total number of the leaves tested × 9) × 100

Control efficacy (%) = (DI of the reference control−DI of the pesticide group)/DI of the reference control × 100

Field experiments: Rice cultivar Boyou 680 was also planted in a field located in the suburb of Nanning City, Guangxi, China in 2015. The experimental field was known to be naturally infested with *Xoc*. The field was divided into twelve plots and each plot had an area of 20 m^2. GA (300 mg · L^{-1}), 20% thiodiazole-copper suspension concentrate(570 mg · L^{-1}) and water (control) were sprayed on rice leaf in the early occurrence of disease. The volume of water delivered was 750 L · hm^{-2}. The application of the bactericide was performed three times at a 10-day-interval. Each treatment consisted of four replicates. No other bactericides were sprayed to the experimental field. Fertilizers were used in accordance with technical standard of agricultural production. The disease severity was recorded on the first day the bactericide was performed and in 10 days after the last application of bactericide. Effect of bactericide on rice bacterial leaf streak was evaluated based on more than 150 rice plants collected randomly from each

plot. The disease severity and DI described earlier were separately investigated. The field control efficacy of bactericide was evaluated using the following formula:

Field control efficacy (%) = [1 − (DI of control at the 1st application × DI of treatment 10 d after the last application)/(DI of treatment at the 1st application × DI of control 10 d after the last application)] × 100

1.9 Statistical analysis

Data were subjected to analysis of variance using SAS software (version 6.08; SAS Institute, Cary, NC). Mean comparisons were conducted using a least significant difference (Fisher's LSD) test at $P = 0.05$. Standard error and LSD results were recorded.

2 Results and Analysis

2.1 Isolation and purification of an antibacterial compound from methanol extract of *S. lineare*

The inhibitory effect of solvent extract from methanol extract of *S. lineare* on *Xoc* varied with solvents. Ethyl acetate extract showed the most effective antibacterial activity against *Xoc* in the test, with an inhibitory diameter of 32.00 mm, followed by N-butanol extract, with an inhibitory diameters of 18.67 mm. Inhibitory diameters of petroleum ether layer, chloroform layer and distilled water layer were 12.67, 15.00 and 16.00 mm, respectively. Therefore, the antibacterial substance of methanol extract of *S. lineare* was mainly in ethyl acetate layer.

The ethyl acetate extract was further separated by silica gel column chromatography. A total 123 fractions were obtained. These fractions were merged into eighteen fractions according to the test of TLC. The fractions of F2 to F9, which had antibacterial activity against *Xoc* , were combined and then purified through a silica gel column again. A total 74 fractions were obtained, and were merged into nine fractions according to the test of TLC. Fraction of F1 to F5, which showed antibacterial activity against *Xoc*, was further separated through a gel filtration chromatography using methanol. The white crystals, which was obtained from 100% methanol and tentatively called Compound **A**, showed inhibitory activity on the growth of *Xoc*.

2.2 Structure identification of monomeric compound

Compound **A** was white acicular crystal, easily dissolvable in methanol, and had a formula of $C_7H_6O_5$ as determined from the electrospray ionization mass spectrometry, the Rf value in TCL plate was the same as that of gallic acid. The spectral data of the Compound **A** were as follows: ^{13}C NMR (Fig. 1) (pyridine, 150.95 MHz): δ 170.0 (−COOH), 147.9 (C-3, 5), 140.8 (C-4), 122.8 (C-1), 110.9 (C-2, 6); ^{1}H NMR (Fig. 2) (pyridine, 600.24 MHz): δ 7.22 (2H, s, H-2, 6). According to the references (Ahmed et al, 2003; Eldahshan, 2011; Sushma et al, 2013), the Compound **A** was identified as 3,4,5- Trihydroxybenzoic acid, namely gallic acid, the chemical structure of the Compound **A** was shown in Fig. 3.

2.3 Antibiotic spectrum of GA

The growth of all test plant-pathogenic bacteria was inhibited by GA atconcentration 30 mg · mL^{-1}. The test bacteria, including *X. campestris* pv. *pruni*, *X. oryzae* pv. *oryzicola*, *X. oryzae* pv. *oryzae* and *X. axonopodis* pv. *citri*, were most strongly inhibited by GA, with inhibition zones width of 25.33, 24.00, 22.33 and 20.67 mm, respectively (Table 1). *Pseudomonas syringae* pv. *glycinea* and *P. syringae* pv. *tomato* showed the least sensitive to GA, with inhibition zones width of 11.00 mm and 8.68 mm ,respectively (Table 1). At concentration of 30 mg · mL^{-1}, GA could also weakly inhibit the growth of eleven plant-pathogenic fungi, including *Phytophthora nicotianae*, *Penicillium digitatum*, *Streptobotrys streptothrix*, *Pythium aphanidermatum*, *Pestalotiopsis mangiferae*, *Curvularia lunata*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *niverum*, *F. oxysporum* f. sp. *nicotianae*, *Botrytis cinerea* and *Sclerotium rolfsii* (Table 2). Growth inhibition exceeding 50% by GA was only observed in *Phytophthora nicotianae* and *Penicillium digitatum*. The growth of *Botrytis cinerea* and *Sclerotium rolfsii* was slightly inhibited by GA, with

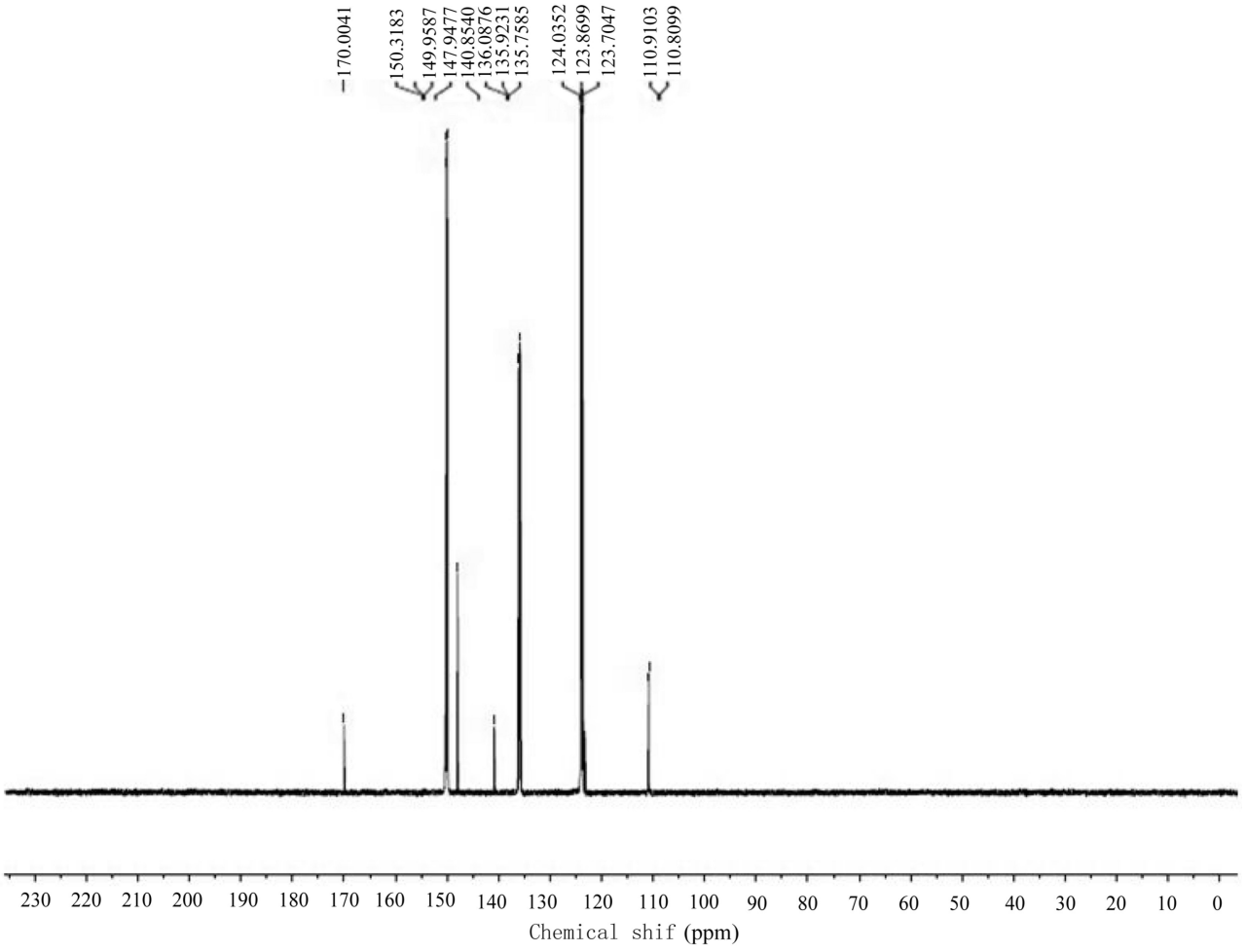

Fig. 1 ^{13}C NMR of the Compound **A**

Table 1 Antibacterial activity of gallic acid against plant pathogenic bacteria

Plant pathogenic bacteria (strain)	Diameter of inhibition zone (mm)
Xanthomonas campestris pv. *pruni* (*Xcp* 112)	25.33±0.22a
X. oryzae pv. *oryzicola* (*Xoc* XD1109)	24.00±1.41a
X. oryzae pv. *oryzae* (*Xoo* 168)	22.33±1.00ab
X. axonopodis pv. *citri* (*Xac* 216)	20.67±0.47ab
Ralstonia solanacearum (*Rs* 862)	18.00±2.02b
Pseudomonas syringae pv. *glycinea* (*Psg* 631)	12.33±0.29c
P. syringae pv. *tomato* (*Pst* 438)	11.00±0.90cd
Pectobacterium carotovora subsp. *carotovora* (*Pcc* 003)	8.68±0.34d

Note: Different small letters indicate significant differences (P = 0.05, Fisher's LSD test). The same below.

growth inhibition 4.58% and 1.96%, respectively. But GA could not inhibit the growth of *Phytophtora cactorum*, *Corynespora cassiicola*, *Magnaporthe grisea*, *Alternaria musae* and *Phyllosticta nicotianae*.

2.4 Control efficacy of gallic acid on the disease

Potted experiment: The results (Table 3) in a greenhouse experiment showed that gallic acid could significantly reduce disease index of rice bacterial leaf streak relative to the water control group. Gallic acid at 200, 300 and 400 mg · L^{-1} provided 63.55%, 71.38% and 77.39% control efficacy respectively. The control efficacy of gallic acid at 200 mg · L^{-1} was lower than that of thiodiazole-copper at 570 mg · L^{-1}, but the control efficacy of gallic acid at 400 mg · L^{-1} was significantly

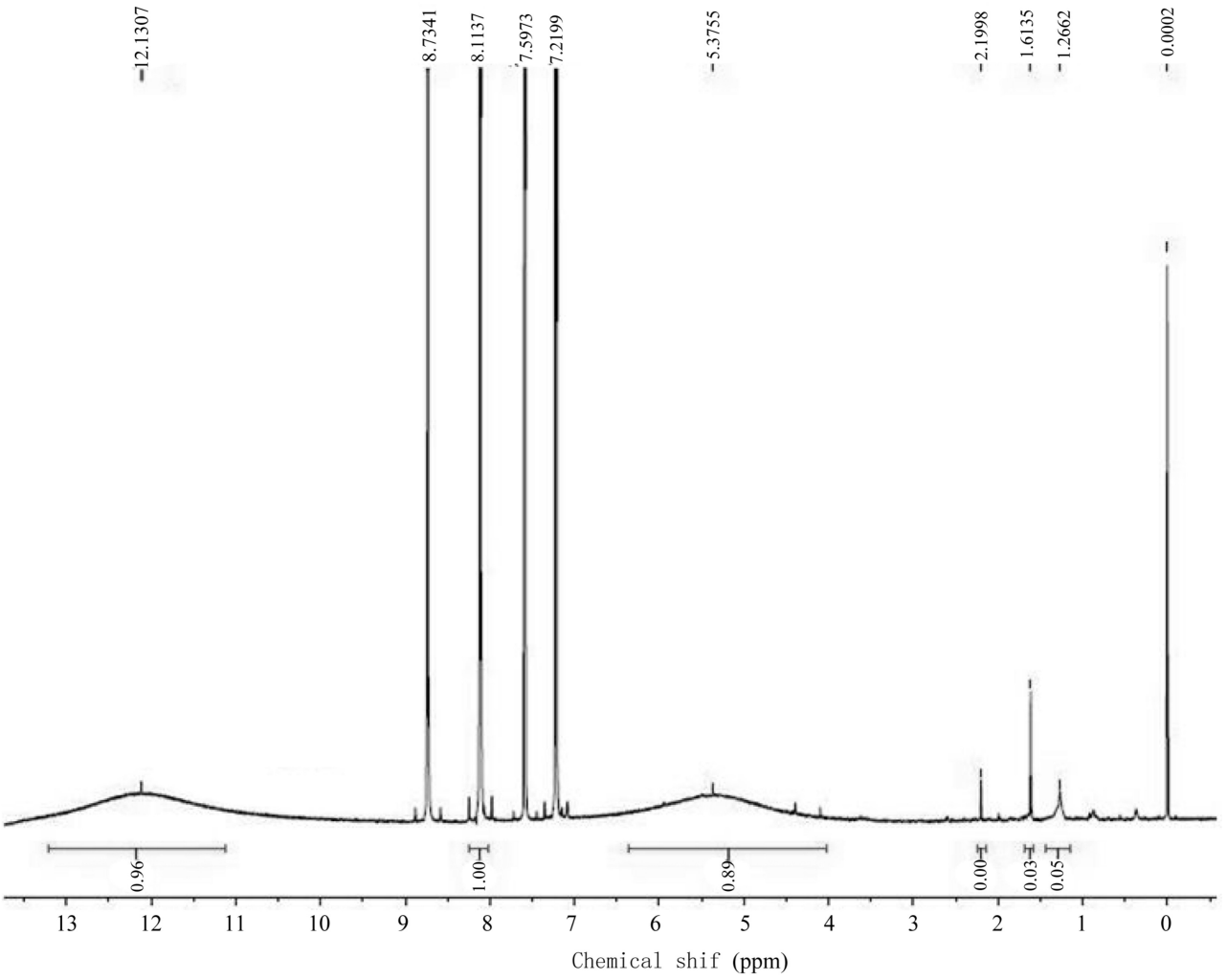

Fig. 2　^{1}H NMR of the Compound **A**

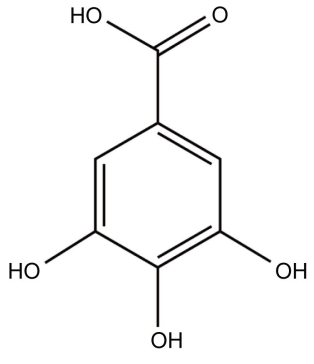

Fig. 3　Chemical structure of gallic acid

higher than that of thiodiazole-copper at 570 mg · L^{-1}. Therefore, gallic acid at concentration 300 mg · L^{-1} was used for field experiment.

Field experiment: Gallic acid could effectively reduce disease index of rice bacterial leaf streak relative to the water control in field. The control efficacies of GA (300 mg · L^{-1}) and 20% thiodiazole-copper suspension concentrate (570 mg · L^{-1}) on rice bacterial leaf streak were 64.62% and 63.37% respectively (Table 4). The control efficacies of GA were similar to that of thiodiazole-copper.

3　Discussion

A compound, which could significantly inhibit the growth of *Xanthomonas oryzae* pv . *oryzicola* (*Xoc*) *in*

Table 2 Antifungal activity of gallic acid against plant pathogen fungi

Plant pathogen fungi (strain)	Inhibition ratio (%)
Phytophthora nicotianae (*Phytn* 169)	56.34±0.96a
Penicillium digitatum (*Penid* 016)	54.73±3.18a
Streptobotrys streptothrix (*Streps* 147)	38.00±0.00b
Pythium aphanidermatum (*Pytha* 013)	29.86±2.40bc
Pestalotiopsis mangiferae (*Pestm* 007)	28.17±2.39bcd
Curvularia lunata (*Curvl* 068)	20.00±0.00cde
Rhizoctonia solani (*Rhizs* 002)	19.02±2.82de
Fusarium oxysporum f. sp. *niverum* (*Foniv* 185)	16.71±2.70ef
F. oxysporum f. sp. *nicotianae* (*Fonic* 239)	10.59±1.28f
Botrytis cinerea (*Botrc* 054)	4.58±1.25g
Sclerotium rolfsii (*Scler* 480)	1.96±4.20g
Phytophtora cactorum (*Phytc* 015)	0.00 h
Corynespora cassiicola (*Coryc* 026)	0.00 h
Magnaporthe grisea (*Magng* 047)	0.00 h
Alternaria musae (*Alterm* 142)	0.00 h
Phyllosticta nicotianae (*Phyln* 065)	0.00 h

Table 3 Control efficacy of gallic acid on rice bacterial leaf streak in potted experiment

Treatment	Concentration ($mg \cdot L^{-1}$)	Disease index	Control efficacy (%)
Gallic acid	200	20.49±0.89b	63.55±1.32c
	300	16.09±0.12c	71.38±0.21b
	400	12.70±0.62d	77.39±0.96a
Thiodiazole-copper	570	16.88±0.50c	69.97±0.83b
Water(CK)	—	56.23±0.20a	—

Note: The values are means of four replicates ± standard errors. Columns with the same letters are not significantly different based on Fisher's LSD test ($P<0.05$). The same below.

vitro, was isolated from *S. lineare* by liquid-liquid extraction, silica gel column chromatography and gel column chromatography in this study. Based on ^{1}H and ^{13}C NMR data, this compound was identified as gallic acid.

Gallic acid has been applied to many fields. Ma et al (1994) found that GA not only shows the antioxidant activity, but also has stability, so it is widely used in the clinical experiment and food industry. Tumors, treated with GA, showed cytotoxic activity against cancer cells, without harming normal cells (Bajpai & Patil, 2008). GA can be used to produce pyrogallic acid as a fresh agent to preserve food (Cheng et al, 1994). GA can be also used in food packaging, because a bio-based multilayer packaging film with GA as the oxygen scavenger (Pant et al, 2017). At present, there are no report on application of GA to control plant diseases. The result of this research showed that gallic acid could significantly decrease the incidence of rice bacterial leaf streak at concentration of 300 $mg \cdot L^{-1}$ in field, the control efficacy of GA at 300 $mg \cdot L^{-1}$ on rice bacterial leaf streak in field was similar to that of 20% thiodiazole-copper suspension concentrate (570 $mg \cdot L^{-1}$). Therefore, gallic acid has the potential to be further developed as a bactericide against rice bacterial leaf streak.

Table 4 Control efficacy of gallic acid on rice bacterial leaf streak in field experiment

Treatment	Concentration ($mg \cdot L^{-1}$)	Disease index on the first day the bactericide was performed	Disease index at 10 days after the last application	Control efficacy (%)
Gallic acid	300	6.40±1.17a	12.33±1.36b	64.62±1.76a
Thiodiazole-copper	570	5.61±0.42a	11.29±0.38b	63.37±0.78a
Water(CK)	—	4.39±0.44a	24.12±0.54a	—

Sedum lineare is mainly used for roof greening. Feng et al (2010) found that *S. lineare* presented a simple but practical energy balance model, which could dissipate 99.1% of the total heat gain of an extensive green roof. *S. lineare* have better temperature reduction effects than purple/red leafed plants and have temperature reduction effectiveness (Liu et al, 2012). The extracts of *S. lineare* can be used in medicine and related applications. Liao et al (2011) found that *S. lineare* has

obvious anti-inflammatory effect. δ-Amyrone, a compound from *S. lineare*, is a bioactive agent which possesses anti-inflammatory effects (Niu et al, 2014). The ethyl acetate extracts, *n*-butyl alcohol extracts and total flavone extracts of *S. lineare* have the antitumor activity (Chen et al, 2011). We found that GA form *S. lineare* could control the rice bacterial leaf streak. Therefore, *S. lineare* presents widely used in many fields.

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