Research Article

Analysis of Antifungal Components in the Galls of *Melaphis chinensis* and Their Effects on Control of Anthracnose Disease of Chinese Cabbage Caused by *Colletotrichum higginsianum*

Ping-Chung Kuo,¹ Ting-Fang Hsieh,² Mei-Chi Lin,¹ Bow-Shin Huang,¹ Jenn-Wen Huang,³ and Hung-Chang Huang⁴

¹Department of Biotechnology, National Formosa University, Yunlin 632, Taiwan

²Floriculture Research Center, Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan, Yunlin 646, Taiwan

³Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan

⁴Plant Pathology Division, Taiwan Agricultural Research Institute, Council of Agriculture, Executive Yuan, Wufeng, Taichung 413, Taiwan

Correspondence should be addressed to Ping-Chung Kuo; pcckuo@nfu.edu.tw and Ting-Fang Hsieh; tfhsieh@tari.gov.tw

Received 8 July 2014; Revised 4 September 2014; Accepted 15 September 2014

Academic Editor: Hasan Uslu

Copyright © 2015 Ping-Chung Kuo et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Fungal pathogens caused various diseases which resulted in heavy yield and quality losses on plants of commercial interests such as fruits, vegetables, and flowers. In our preliminary experimental results, the methanol extracts of four species of medicinal plants *Melaphis chinensis*, *Eugenia caryophyllata*, *Polygonum cuspidatum*, and *Rheum officinale* possessed antifungal activity to causal agent of cabbage anthracnose, *Colletotrichum higginsianum*. Thus it was conducted to identify and quantify the chemical constituents in these herbs and to assess the antifungal effects of these compounds. Among the tested principles, the indicator compound methyl gallate from *M. chinensis* was the most effective one against the conidial germination. In addition, it exhibited significant effects of controlling anthracnose disease of Chinese cabbage caused by *C. higginsianum* PA-01 in growth chamber. These results indicate that *M. chinensis* may be potential for further development of plant-derived pesticides for control of anthracnose of cabbage and other cruciferous crops.

1. Introduction

There are numerous reports indicating that tissues of some plant species contain antifungal substances, including rhizomes of *Curcuma longa* [1], seeds of *Cassia tora* [2], stem/leaves and flowers of *Lavandula stoechas* [3], and others. For example, seeds of mustard (*Brassica juncea* cv. Bau Sin) are rich in glucosinolate and enzymatic hydrolysis of this compound resulted in the release of allyl isothiocyanate that is highly toxic to *Rhizoctonia solani* Kühn AG-4, causal agent of root rot of cabbage [4]. Some plant species with antifungal properties are also used as medicinal plants. For example, galls of *Melaphis chinensis* [5, 6] and leaves of *Aloe vera* [7] contained toxic substances against plant pathogenic fungi.

The *n*-hexane fraction of a cinnamon (*Cinnamomum cassia*) extract exhibited significant inhibition on mycelia growth of *R. solani* [8]. Various essential oils also displayed significant antifungal activity, such as those from *Hypericum linarioides* [9], *Pistacia lentiscus* [10], *Metasequoia glyptostroboides* [11], and *Silene armeria* [12]. The essential oils of cinnamon leaves (*Cinnamomum zeylanicum*) and clove buds (*Eugenia caryophyllata*) also showed highly antifungal activity against *Botrytis cinerea* [13]. Chu et al. reported that the aqueous extracts of *Coptis chinensis* (goldthread), *Polygonum cuspidatum* (Japanese knotweed), *Cinnamomum cassia* (cinnamon), *Rheum officinale* (Chinese rhubarb), *Polygonum multiflorum*, and *Eugenia caryophyllata* (clove) showed inhibitory effects to conidial germination of *Oidium murrayae* [14].

Water-soluble extracts of clove completely inhibited the conidial germination and mycelial growth of *C. higginsianum* at the concentration of 1% (w/v). In addition, clove oil and eugenol were equally effective in reducing disease severity of anthracnose caused by this pathogen in greenhouse [15]. Although the antifungal activities of various plants were extensively reported, there were relatively few studies regarding the antifungal principles in the plant extracts.

Colletotrichum species are important fungal pathogens causing anthracnose disease of numerous economically important crops, including legumes, ornamentals, vegetables, and fruit trees [16–22] and thus are responsible for severe yield losses of cabbage crops in commercial fields in Taiwan [23]. Although these diseases could be successfully controlled by the synthetic chemical fungicides, the utilization of synthetic fungicides led to the development of resistance and environment pollution. The biological control of plant diseases which is recognized as use of metabolites from the natural source is an eco-friendly resolution [24]. In our preliminary experimental results (Table 1), forty herbal extracts were examined for their antifungal activity against *C. higginsianum* PA-01. Most of them displayed inhibition of the fungus and among the tested methanol extracts, four species of medicinal plants, *Melaphis chinensis*, *Eugenia caryophyllata*, *Polygonum cuspidatum*, and *Rheum officinale*, exhibited the inhibitory percentages between 80.64 ± 3.16 and $91.92 \pm 3.00\%$ at the tested concentration ($1250 \mu\text{g/mL}$). The experimental data indicated that these extracts possessed antifungal activity to causal agent of cabbage anthracnose *C. higginsianum*. However, the chemical nature of the antifungal substances in these Chinese herbs remains unknown. Therefore, the objectives of this study were to identify the compounds and their antifungal activity in four species of medicinal plants. In addition, the indicator compounds were used as standards to quantitatively analyze these medicinal plants with the aid of high performance liquid chromatography (HPLC) and the validation examinations were performed to confirm that these methods were precise and reliable for quality evaluation. Thus they could be utilized to control the quality of herbal preparations to ensure their antifungal activities. Moreover, their effects of controlling anthracnose disease of Chinese cabbage caused by *Colletotrichum higginsianum* PA-01 in growth chamber were also examined.

2. Materials and Methods

2.1. General Procedure. All the solvents including the HPLC-grade methanol were purchased from Merck KGaA (Darmstadt, Germany). The chemical structures of the indicator compounds were identified by comparison of their spectroscopic and physical data with those reported in the literature. Their purities were better than 99.0% as determined by HPLC. Plant materials were extracted using a Major Science LM-570R shaking incubator. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-20ATseries pumping system equipped with a Shimadzu SPD-20AUV-Vis detector, a Gemini 5u C18 column

($4.6 \text{ mm} \times 250 \text{ mm}$, $5 \mu\text{m}$), and a SIL-10AF autosampling system.

2.2. Fungal Pathogen and Plant Materials. Two isolates (PA-01 and PA-19) of *C. higginsianum* were used in this study. They were isolated from diseased leaves of cabbage (*Brassica rapa* L. Chinese group) grown in Yunlin, Taiwan. The cultures were maintained on potato dextrose agar (PDA, Difco, USA). Dry powders of all the examined medicinal herbs were purchased from herbal stores in Yunlin, Taiwan. All the purchased materials for the experiments were authenticated by Dr. T. F. Hsieh and the voucher specimens (PCKuo_TFHsieh_201001-201040) were deposited in the herbarium of Department of Biotechnology, National Formosa University, Yunlin, Taiwan. Seeds of Chinese cabbage (*B. rapa* L.) were put on a number 1 filter paper (9 cm in diameter, Toyo Roshi Co., Japan) moistened in water and kept in a Petri dish at room temperature ($22\text{--}25^\circ\text{C}$) for 1 day. The germinated seeds were sown in peat moss in plastic pots, 128 cells/tray, and 1 seed/cell. After one week, individual seedlings were transplanted to plastic pots (18 cm in diameter) filled with Stender peat substrates (Stender AG, Germany), 3 plants/pot, and kept in a greenhouse for four weeks with daily watering.

2.3. Effect of the Methanol Extracts on Conidial Germination of *C. higginsianum*. The methanol extracts were tested for inhibition of conidial germination of *C. higginsianum* PA-01 according to the method of Lee and Dean [25]. The isolate was grown on oatmeal agar (50 g/L) at 25°C under continuous fluorescent light. Conidia were harvested from 7- to 10-day-old cultures and the solution was filtered to collect conidial suspension. Ten microliters of conidial suspension ($\sim 10^5$ conidia/mL) was mixed with ten microliters of different extracts. The cultures were placed in a moistened plastic box, incubated at 25°C for 24 h, and examined for germination of conidia under a compound microscope. There were six replicates for each sample (100 conidia/replicate). Sterile distilled water was used as negative control and azoxystrobin was used as positive control. Inhibition rate of conidia for each treatment was calculated by

$$\begin{aligned} & \text{Inhibition (\%)} \\ &= \left[1 - \frac{(\text{conidial germinated with tested compound})}{(\text{conidial germinated in control})} \right] \\ & \times 100\%. \end{aligned} \quad (1)$$

2.4. Extraction and Fractionation of Medicinal Plants. The galls of *M. chinensis* (60.0 g) were extracted with methanol under reflux ($0.5 \text{ L} \times 5 \times 8 \text{ h}$), and the crude extracts were concentrated *in vacuo* to give a brown syrup (MCR, 50.0 g). The crude extract was partitioned between ethyl acetate and water to afford ethyl acetate solubles (MCRE, 46.0 g) and water extracts (MCRW, 4.0 g), respectively. The buds of *E. caryophyllata* (30.0 g) were extracted with methanol under reflux ($0.2 \text{ L} \times 5 \times 8 \text{ h}$), and the crude extracts were concentrated to give a brown syrup (EC, 7.0 g). The crude

TABLE 1: The preliminary antifungal screening of the different herbal extracts on conidial germination of *Colletotrichum higginsianum* PA-01.

Sample	Inhibition percentage (%) ^a	Sample	Inhibition percentage (%) ^a
<i>Anemarrhena asphodeloides</i>	6.55 ± 2.73	<i>Lithospermum erythrorhizon</i>	8.15 ± 4.32
<i>Arctium lappa</i>	3.99 ± 3.14	<i>Lycium barbarum</i>	4.90 ± 1.72
<i>Cassia angustifolia</i>	— ^b	<i>Melaphis chinensis</i>	89.86 ± 2.00
<i>Cassia tora</i>	12.67 ± 2.71	<i>Morus alba</i>	—
<i>Carthamus tinctorius</i>	—	<i>Paeonia lactiflora</i>	4.30 ± 3.06
<i>Cinnamomum cassia</i>	27.30 ± 3.89	<i>Polygala tenuifolia</i>	—
<i>Crataegus pinnatifida</i>	5.89 ± 2.32	<i>Polygonum cuspidatum</i>	80.64 ± 3.16
<i>Cuscuta chinensis</i>	15.18 ± 1.83	<i>Prunella vulgaris</i>	36.44 ± 4.63
<i>Epimedium brevicornum</i>	5.89 ± 3.02	<i>Prunus armeniaca</i>	2.92 ± 3.66
<i>Equisetum hyemale</i>	10.75 ± 2.86	<i>Pueraria lobata</i>	3.99 ± 2.58
<i>Eucommia ulmoides</i>	2.43 ± 1.60	<i>Rheum officinale</i>	91.92 ± 3.00
<i>Eugenia caryophyllata</i>	87.37 ± 4.74	<i>Salvia miltiorrhiza</i>	8.81 ± 2.28
<i>Forsythia suspensa</i>	15.42 ± 4.32	<i>Scrophularia ningpoensis</i>	9.73 ± 2.64
<i>Gardenia jasminoides</i>	3.54 ± 2.59	<i>Scutellaria barbata</i>	7.25 ± 1.05
<i>Gentiana scabra</i>	6.06 ± 1.55	<i>Smilax glabra</i>	8.08 ± 3.46
<i>Hedyotis diffusa</i>	0.51 ± 1.94	<i>Sophora flavescens</i>	31.47 ± 3.83
<i>Houttuynia cordata</i>	—	<i>Sophora tonkinensis</i>	27.10 ± 4.04
<i>Isatis indigotica</i>	4.33 ± 2.10	<i>Taraxacum mongolicum</i>	5.37 ± 2.19
<i>Leonurus japonicus</i>	14.85 ± 2.99	<i>Zingiber officinale</i>	24.62 ± 4.51
<i>Ligusticum chuanxiong</i>	8.21 ± 3.62	<i>Ziziphus jujuba</i>	28.01 ± 4.88

^aPercentage of inhibition at 1250 µg/mL (800X dilution) concentration. (*n* = 6). ^bNo inhibition was found.

extract was partitioned between ethyl acetate and water to afford ethyl acetate solubles (ECE, 5.5 g) and water extracts (ECW, 1.5 g), respectively. The roots of *P. cuspidatum* (100.0 g) were extracted with methanol under reflux (0.3 L × 5 × 8 h), and the crude extracts were concentrated to give a brown syrup (PC, 8.0 g). The crude extract was partitioned between chloroform and water to afford chloroform solubles (PCC, 1.7 g) and water extracts (PCW, 6.3 g), respectively. The roots of *R. officinale* (100.0 g) were extracted with methanol under reflux (0.3 L × 5 × 8 h), and the crude extracts were concentrated to give a brown syrup (RO, 27.0 g). The crude extract was partitioned between chloroform and water to afford chloroform solubles (ROC, 2.8 g) and water extracts (ROW, 24.2 g), respectively.

2.5. Purification and Identification of Indicator Compounds. The methods for purification and identification of indicator compounds in the four medicinal plants were described as follows.

(I) *M. chinensis*. The ethyl acetate soluble fraction (MCRE, 40.0 g) of the crude extract was applied to a silica gel column and then eluted with chloroform and step gradient of ethyl acetate (10:1 to 1:1, v/v) to yield 9 fractions. Fraction 3 was subjected to silica gel column chromatography eluted with *n*-hexane and acetone (10:1, v/v) to yield methyl gallate (**2**) (10.5 g). Fraction 8 was further resolved on a silica gel column eluted with chloroform and acetone (5:1) to give gallic acid (**1**) (2.3 g).

(II) *E. caryophyllata*. The ethyl acetate soluble fraction (ECE, 5.5 g) of the crude extract was purified with silica gel column chromatography and eluted with *n*-hexane and step gradient of ethyl acetate (20:1 to 1:1, v/v) to yield 5 fractions. Fraction 2 was further purified on a silica gel column eluted with chloroform and ethyl acetate (20:1, v/v) to give eugenol (**3**) (250.0 mg).

(III) *P. cuspidatum* and *R. officinale*. For *P. cuspidatum*, the chloroform soluble fraction (PCC, 1.7 g) of the crude extract was purified with silica gel column chromatography and eluted with chloroform and step gradient of ethyl acetate (50:1 to 1:1, v/v) to yield 9 fractions. Fraction 2 was further recrystallized with chloroform and ethyl acetate to afford physcion (**6**) (25.0 mg). Fraction 6 was further resolved on a silica gel column eluted with chloroform and step gradient of methanol (100:1 to 1:1, v/v) to yield emodin (**4**) (30.0 mg). For *R. officinale*, the chloroform soluble fraction (ROC, 2.8 g) of the crude extract was purified with silica gel column chromatography and eluted with chloroform and step gradient of methanol (300:1 to 1:1, v/v) to afford 11 fractions. Fraction 2 was further purified with the assistance of silica gel column chromatography eluted with *n*-hexane and acetone (100:1, v/v) to give chrysophanol (**5**) (28.0 mg).

2.6. Chromatography. The six indicator compounds used in chromatographic analysis were gallic acid (**1**) and methyl gallate (**2**) from *M. chinensis*, eugenol (**3**) from *E. caryophyllata*, emodin (**4**) and physcion (**6**) from *P. cuspidatum*, and chrysophanol (**5**) from *R. officinale*. The analytic conditions

for these chemical constituents were determined by HPLC according to the reported methods in the literature [26–28].

2.7. Preparation of Standard Solutions, Calibration Curves, and Validation of the Analytical Methods. The standard solutions and calibration curves for the six indicator compounds were prepared according to the methods reported in the literature [29]. The reproducibility and precision of detection were measured by repeatedly injecting a ready-made sample pool and expressed as the relative standard deviation of the results. To determine the variance of samples within a day, the same samples were tested at different times within the day. The variance between days was determined by assaying the spiked samples over three consecutive days at the same time each day. The limit of detection (LOD) was determined as the lowest detectable concentration with acceptable accuracy and precision and three times above the noise level ($S/N \geq 3$). The recovery of the indicator compounds was evaluated using three different concentrations covering the linear range of the standard curve and the peak heights were compared to the standard compounds to calculate the recovery data.

2.8. Effect of the Methanol Extracts, Partially Purified Fractions, and Indicator Compounds on Conidial Germination of *C. higginsianum*. Each of the four methanol extracts, partially purified fractions, and the six indicator compounds from the plant extracts were tested for inhibition of conidial germination of *C. higginsianum*, isolates PA-01 and PA-19, as described previously [25].

2.9. Effect of Gallic Acid and Methyl Gallate on Control of Anthracnose Disease of Chinese Cabbage Caused by *C. higginsianum* PA-01 in Growth Chamber. To determine the effect of indicator compounds, gallic acid, and methyl gallate, on control of anthracnose disease of Chinese cabbage, each dilution of gallic acid and methyl gallate derived from galls of *M. chinensis* with the concentration of 125, 250, 500, and 1000 $\mu\text{g/mL}$ was sprayed on 3-week-old Chinese cabbage plants until running water one day prior to the inoculation of *C. higginsianum* PA-01. Plants sprayed with sterile distilled water were used as controls. There were three replicates (pots) for each treatment. Conidial suspensions of *C. higginsianum* were inoculated on each plant at 8 mL/plant and 10^5 conidia/mL, using a compressed air-sprayer (SIL-AIR, Werther International, Italy). All pots were placed in moist plastic bags and kept in a growth chamber at 24°C under 12 h diurnal illumination. The plastic bags were removed after one-day incubation and the plants were examined for lesion number and infection area in 3 cm diameter of leaf spot at 5, 7, and 9 days after inoculation.

2.10. Statistical Analysis. Data collected from all the experiments in this study were analyzed for statistical significance using analysis of variance (ANOVA). Means of treatments in each experiment were separated using Duncan's multiple range tests. The analytical results are expressed as mean $\pm$ standard deviation (SD). Relative standard deviations (RSDs) were calculated from those values. In addition, the mean

values of lesion number and lesion area on infection leaves were analyzed by the least significant difference (LSD) test.

3. Results and Discussion

3.1. Antifungal Activities of the Crude Extracts. The antifungal effects of the forty herbal extracts on conidial germination of *C. higginsianum* PA-01 are displayed in Table 1. Among the examined samples, four species of medicinal plants, including *Melaphis chinensis*, *Eugenia caryophyllata*, *Polygonum cuspidatum*, and *Rheum officinale*, displayed significant antifungal activity against *C. higginsianum* PA-01. Thus these four extracts were selected as the targets of developing new botanical pesticides. Although the synthetic fungicides successfully controlled the plant diseases sometimes, they also contributed to increasing the population of fungicide-resistant pathogens [30]. Natural plant metabolites are generally considered as safe to the humans and environment since these chemical compounds are easily decomposed in the soil and would not exhibit long-term effects to the environment [31]. Thus more and more reports were focused on the development of new plant-derived pesticide preparations recently [1, 4, 15, 32, 33], but comparatively few studies related to the antifungal principles in the bioactive extracts were completed. These new preparations would be hopeful to reduce the damage caused by traditional synthetic fungicides and in the meanwhile to suppress the disease development effectively. Detailed chemical analysis of the constituents in the plant extracts is helpful to confirm the active compounds and control the quality of the plant-derived pesticide preparations.

3.2. Identification of Indicator Compounds in the Medicinal Plant Extracts. The indicator compounds (Figure 1), including gallic acid (1) [34] and methyl gallate (2) [35] from the galls of *M. chinensis*, eugenol (3) [36] from buds of *E. caryophyllata*, emodin (4) [37] and physcion (6) [38] from the roots of *P. cuspidatum*, and chrysophanol (5) [39] from the roots of *R. officinale*, were purified and characterized by comparison of their spectral and physical data with those reported in the literature. The purity of all the indicator compounds except physcion (6) as determined by HPLC was better than 99.0%.

3.3. Optimization of the HPLC Condition and Method Validation. The optimized HPLC analytical conditions for the medicinal plant extracts were designed as displayed in the experimental section. The calibration curve parameters and limits of detection (LOD) for the indicator compounds were displayed in Table 2. The precision of the HPLC method developed was evaluated through the intraday and interday experiments. Among the linear ranges, the RSDs for all the indicator compounds of the intraday and interday precisions were found to be less than 1.62 and 2.61%, respectively (Table 2). The recovery of the indicator compounds was determined by the addition of a sample with known concentration to the standard solution, and the mean recovery rate was found to be in the ranges from 81.12 to 126.33%

FIGURE 1: Chemical structures of the indicator compounds 1–6.

with satisfactory RSDs in the ranges between 0.09 and 3.26% (Table 2).

In the present study, the indicator compounds in *M. chinensis*, *E. caryophyllata*, *C. cassia*, *P. cuspidatum*, and *R. officinale* had been extracted and purified. The indicator compounds were further used as standards to quantitatively analyze these traditional Chinese medicines with the aid of HPLC and the validation examinations were carried out to confirm that these methods were precise and reliable for quality evaluation. In the development of the HPLC method for the quantitative determination of indicator compounds, several solvent systems and separation columns were evaluated and compared. Detection wavelength was also optimized in this work. The maximum number and the heights of the peaks of the constituents were obtained and the baseline of chromatogram was stable. The reproducibility of the analytical method was performed and the results showed that it was satisfactory with the RSDs below 3.0% for any of the indicator compounds (data not shown). The precision and recovery tests all displayed that the established HPLC chromatographic methods were valid for the quantitative determination of the indicator compounds and also convenient and feasible as tools for species authentication and quality assessment of the herbal raw materials.

3.4. Quantitative Determination of Indicator Compounds in the Medicinal Plant Extracts. The developed HPLC chromatographic analytical methods were applied to assess the contents of the indicator compounds in the extracts of corresponding plant materials and the data were displayed in Table 3. The contents of methyl gallate (2) and eugenol

(3) in the ethyl acetate soluble fractions of methanol extracts of *M. chinensis* and *E. caryophyllata*, respectively, were more than 30% and they indicated that these constituents were the major component in the plant extracts. In contrast, emodin (4), chrysophanol (5), and physcion (6) were less than 5% in the methanol extracts of *P. cuspidatum* and *R. officinale*. The reproducibility of the analytical results was satisfactory with the RSDs below 3.53% for all the examined indicator compounds.

3.5. Antifungal Activities of the Methanol Extracts, Partially Purified Fractions, and Indicator Compounds. The antifungal effects of the extracts and fractions on conidial germination of *C. higginsianum* PA-01 and PA-19 are displayed in Table 4. Most of the crude extracts and low polarity fractions displayed inhibitions of the conidial germination of the fungal pathogen. Among the tested samples, the ethyl acetate fraction of the methanol extracts of *M. chinensis* exhibited the most significant antifungal activities towards *C. higginsianum* PA-01 and PA-19 with the IC_{50} values of 236.6 and 191.4 $\mu\text{g/mL}$, respectively. The antifungal effects of the indicator compounds 1–6 and the reference compound azoxystrobin were displayed in Table 5. Compounds 1–5 all exhibited the inhibitory effects against *C. higginsianum* PA-01 with the IC_{50} values less than 850.3 $\mu\text{g/mL}$, and comparatively compounds 1–4 and 6 showed the significant inhibition of the conidial germination of *C. higginsianum* PA-19 with the IC_{50} values ranging from 22.1 to 1259.0 $\mu\text{g/mL}$, respectively. The major component methyl gallate (2) in the most active fraction (MCRE) displayed the most significant antifungal effects with the IC_{50} values of 40.2

TABLE 2: Calibration curve parameter, limits of detection (LOD), precision, and recovery for the indicator compounds.

Compound	Calibration curve	Correlation coefficients (r^2)	Linear range ($\mu\text{g/mL}$)	LOD ($\mu\text{g/mL}$)	Concentration ($\mu\text{g/mL}$)	Intraday precision Mean $\pm$ SD (RSD %)	Interday precision (RSD %)	Spiked concentration ($\mu\text{g/mL}$)	Recovery (%)	RSD (%)
1	$y = 12112x + 33065$	0.9995	2.49–498.00	0.180	9.96	9.02 $\pm$ 0.06 (0.63)	8.76 $\pm$ 0.23 (2.61)	6.58	118.02 $\pm$ 3.85	3.26
					249.00	250.86 $\pm$ 0.48 (0.19)	248.87 $\pm$ 1.63 (0.65)	13.63	105.45 $\pm$ 1.11	1.05
					498.00	497.11 $\pm$ 0.67 (0.14)	493.71 $\pm$ 2.90 (0.59)	26.67	106.78 $\pm$ 1.10	1.10
2	$y = 111797x + 53348$	0.9996	2.50–500.00	0.200	9.96	8.69 $\pm$ 0.14 (1.62)	8.57 $\pm$ 0.13 (1.25)	26.60	126.33 $\pm$ 0.91	0.72
					249.00	252.57 $\pm$ 0.71 (0.28)	251.79 $\pm$ 1.71 (0.68)	120.33	106.75 $\pm$ 0.33	0.31
					498.00	498.75 $\pm$ 3.55 (0.71)	498.75 $\pm$ 2.51 (0.50)	264.79	105.16 $\pm$ 0.43	0.41
3	$y = 12313x + 376$	0.9999	24.50–490.05	0.012	49.01	48.85 $\pm$ 0.13 (0.26)	49.61 $\pm$ 0.70 (1.42)	102.64	108.61 $\pm$ 2.88	2.65
					98.01	98.71 $\pm$ 0.36 (0.36)	98.91 $\pm$ 0.70 (0.71)	124.62	96.98 $\pm$ 0.60	0.62
					490.05	489.95 $\pm$ 0.94 (0.19)	498.99 $\pm$ 7.68 (1.54)	168.50	93.01 $\pm$ 1.20	1.29
4	$y = 83352x - 359078$	0.9998	10.09–50.47	0.010	10.09	10.00 $\pm$ 0.02 (0.21)	10.01 $\pm$ 0.02 (0.19)	10.09	95.59 $\pm$ 0.42	0.44
					25.24	25.40 $\pm$ 0.19 (0.73)	25.81 $\pm$ 0.60 (2.34)	25.24	89.26 $\pm$ 0.13	0.14
					50.47	50.44 $\pm$ 0.33 (0.65)	50.31 $\pm$ 0.31 (0.61)	50.47	90.41 $\pm$ 0.08	0.09
5	$y = 92840x - 376375$	0.9992	9.93–49.66	0.010	9.93	10.33 $\pm$ 0.08 (0.82)	10.31 $\pm$ 0.05 (0.45)	9.93	81.12 $\pm$ 2.15	2.65
					24.83	24.19 $\pm$ 0.14 (0.56)	24.17 $\pm$ 0.12 (0.52)	24.83	99.81 $\pm$ 0.35	0.36
					49.66	49.89 $\pm$ 0.10 (0.19)	50.29 $\pm$ 0.65 (1.30)	49.66	108.67 $\pm$ 0.49	0.45
6	$y = 54687x - 123241$	0.9968	9.75–48.76	0.010	9.75	10.55 $\pm$ 0.09 (0.86)	10.61 $\pm$ 0.08 (0.72)	9.75	88.64 $\pm$ 0.63	0.71
					24.38	23.11 $\pm$ 0.06 (0.26)	23.35 $\pm$ 0.19 (0.81)	24.83	82.00 $\pm$ 0.36	0.44
					48.76	49.24 $\pm$ 0.25 (0.50)	49.76 $\pm$ 0.51 (1.03)	48.76	94.96 $\pm$ 0.74	0.78

TABLE 3: Contents of each indicator compound in the crudes and fractions of the examined herb extracts.

Compound	MCR	MCRE	MCRW	EC	ECE	Samples						RO	ROC	ROW
						ECW	PC	PCC	PCW	RO	ROC			
1	4.84 ± 0.14 (2.90) ^a	5.20 ± 0.17 (3.35)	4.66 ± 0.12 (2.63)	—	—	—	—	—	—	—	—	—	—	—
2	29.57 ± 0.32 (1.08)	36.24 ± 1.28 (3.53)	6.02 ± 0.15 (2.47)	—	—	—	—	—	—	—	—	—	—	—
3	— ^b	—	—	26.12 ± 0.70 (2.68)	43.83 ± 1.15 (2.63)	N.D. ^c	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	0.46 ± 0.01 (1.08)	2.93 ± 0.01 (0.28)	N.D.	2.36 ± 0.00 (0.13)	2.15 ± 0.01 (0.65)	N.D.	—	—
5	—	—	—	—	—	—	N.D.	N.D.	N.D.	3.71 ± 0.01 (0.30)	2.06 ± 0.00 (0.09)	N.D.	—	—
6	—	—	—	—	—	—	0.59 ± 0.01 (1.30)	3.94 ± 0.01 (0.32)	N.D.	1.38 ± 0.01 (0.82)	1.79 ± 0.01 (0.55)	N.D.	—	—

^aThe contents of each compound were presented as mean ± S.D. (RSD %) (% g/g sample). ^bNot determined. ^cNot detectable.

TABLE 4: Antifungal activity of crude extracts and fractions of Chinese medicinal herbs^a.

Sample	<i>C. higginsianum</i> PA-01			<i>C. higginsianum</i> PA-19		
	Concentration ($\mu\text{g/mL}$)	Inhibition of conidial germination (%) ^b	IC ₅₀ ($\mu\text{g/mL}$)	Concentration ($\mu\text{g/mL}$)	Inhibition of conidial germination (%)	IC ₅₀ ($\mu\text{g/mL}$)
MCR	315.63	16.27 $\pm$ 4.88***	699.7	303.13	6.35 $\pm$ 2.95***	673.5
	631.25	59.35 $\pm$ 3.67***		606.25	49.92 $\pm$ 5.87***	
	1262.50	89.86 $\pm$ 2.00***		1212.50	97.32 $\pm$ 2.17***	
MCRE	76.56	8.02 $\pm$ 3.74***	236.6	76.17	18.15 $\pm$ 4.70***	191.4
	153.13	24.83 $\pm$ 3.76***		152.34	43.94 $\pm$ 3.30***	
	306.25	68.64 $\pm$ 2.76***		304.69	75.68 $\pm$ 3.95***	
MCRW	317.19	5.08 $\pm$ 2.63***	918.3	304.69	-0.17 $\pm$ 0.41***	984.4
	634.38	20.07 $\pm$ 4.73***		609.38	4.87 $\pm$ 4.21***	
	1268.75	77.15 $\pm$ 5.99***		1218.75	81.74 $\pm$ 4.04	
EC	318.75	16.47 $\pm$ 6.76***	671.3	310.94	23.74 $\pm$ 4.08***	622.1
	637.50	60.45 $\pm$ 5.66***		621.88	47.06 $\pm$ 3.97***	
	1275.00	87.37 $\pm$ 4.74***		1243.75	94.07 $\pm$ 3.11***	
ECE	314.06	43.46 $\pm$ 5.34***	381.0	303.13	44.78 $\pm$ 5.10***	397.8
	628.13	69.52 $\pm$ 6.19***		606.25	61.85 $\pm$ 4.20***	
	1256.25	94.54 $\pm$ 3.47***		1212.50	99.15 $\pm$ 1.00***	
ECW	340.63	7.81 $\pm$ 3.27***	884.4	304.69	6.73 $\pm$ 5.18**	557.4
	681.25	43.32 $\pm$ 8.70***		609.38	40.67 $\pm$ 6.45***	
	1362.50	82.76 $\pm$ 4.27***		1218.75	76.10 $\pm$ 4.16***	
PC	314.06	0.51 $\pm$ 1.90***	839.6	300.00	0.83 $\pm$ 1.10	864.0
	628.13	22.97 $\pm$ 4.53***		600.00	28.76 $\pm$ 4.58***	
	1256.25	91.92 $\pm$ 3.00***		1200.00	85.95 $\pm$ 3.59***	
PCC	326.56	14.60 $\pm$ 3.31***	718.1	300.00	1.34 $\pm$ 1.38	757.2
	653.13	54.05 $\pm$ 5.72***		600.00	49.33 $\pm$ 5.45***	
	1306.25	98.15 $\pm$ 2.34***		1200.00	88.29 $\pm$ 2.17***	
PCW	326.56	0.68 $\pm$ 3.07***	— ^c	326.56	0.50 $\pm$ 1.21	— ^c
	653.13	5.74 $\pm$ 4.05***		653.13	6.69 $\pm$ 2.62	
	1306.25	42.09 $\pm$ 5.52***		1306.25	12.54 $\pm$ 3.56	
RO	331.25	5.09 $\pm$ 4.71***	925.6	312.50	1.00 $\pm$ 1.17	843.6
	662.50	40.88 $\pm$ 4.78***		625.00	32.27 $\pm$ 3.95***	
	1325.00	80.64 $\pm$ 3.16***		1250.00	86.12 $\pm$ 3.72***	
ROC	318.75	19.69 $\pm$ 4.34***	680.9	309.38	1.34 $\pm$ 1.05	736.6
	637.50	43.41 $\pm$ 4.27***		618.75	49.50 $\pm$ 4.43***	
	1275.00	91.75 $\pm$ 3.75***		1237.50	99.50 $\pm$ 0.84***	
ROW	326.56	0.34 $\pm$ 2.76***	884.1	306.25	0.50 $\pm$ 1.21	984.5
	653.13	37.16 $\pm$ 5.84***		612.50	17.06 $\pm$ 3.40***	
	1306.25	79.29 $\pm$ 3.60***		1225.00	75.59 $\pm$ 3.95***	

^an = 6; ^bmean $\pm$ S.D.; **P < 0.01; ***P < 0.001; ^cIC₅₀ > 1306.25 $\mu\text{g/mL}$ and not determined.

and 22.1 $\mu\text{g/mL}$ towards *C. higginsianum* PA-01 and PA-19 (Figure 2), respectively, compared to the reference synthetic pesticide azoxystrobin (IC₅₀ 0.5 and 0.4 $\mu\text{g/mL}$ against *C. higginsianum* PA-01 and PA-19, resp.). Among the examined samples, most of them displayed significant inhibition of the conidial germination of the pathogen and this indicated that these plant-derived pesticide preparations were promising.

3.6. Effect of Gallic Acid and Methyl Gallate on Control of Anthracnose Disease of Chinese Cabbage Caused by *C. higginsianum* PA-01 in Growth Chamber. Suppression of Chinese cabbage anthracnose by indicator compounds, gallic acid, and methyl gallate was dependent on the concentration, where lesion area (%) and lesion number per 3 cm in diameter of infected leaf were decreased by increasing concentrations of each indicator compound (Table 6). In general, disease

TABLE 5: Antifungal activity of indicator compounds^a.

Compound	<i>C. higginsianum</i> PA-01			<i>C. higginsianum</i> PA-19		
	Concentration ($\mu\text{g/mL}$)	Inhibition of conidial germination (%) ^b	IC ₅₀ ($\mu\text{g/mL}$)	Concentration ($\mu\text{g/mL}$)	Inhibition of conidial germination (%)	IC ₅₀ ($\mu\text{g/mL}$)
1	323.44	20.00 $\pm$ 3.19***	586.4	307.81	34.45 $\pm$ 5.73***	361.6
	646.88	76.19 $\pm$ 4.36***		615.63	81.34 $\pm$ 3.42***	
	1293.75	98.28 $\pm$ 1.68***		1231.25	99.50 $\pm$ 0.84***	
2	19.73	25.81 $\pm$ 5.67***	40.2	9.42	25.96 $\pm$ 3.63***	22.1
	39.45	64.02 $\pm$ 4.34***		18.85	47.56 $\pm$ 3.61***	
	78.91	84.98 $\pm$ 4.03***		37.70	76.05 $\pm$ 2.50***	
3	318.75	13.93 $\pm$ 3.23***	716.5	320.31	34.01 $\pm$ 4.12***	434.5
	637.50	53.13 $\pm$ 4.37***		640.63	79.16 $\pm$ 3.97***	
	1275.00	84.14 $\pm$ 3.96***		1281.25	99.32 $\pm$ 1.23***	
4	310.94	10.40 $\pm$ 2.47***	749.1	325.00	2.67 $\pm$ 2.41	759.9
	621.88	43.82 $\pm$ 4.53***		650.00	60.20 $\pm$ 2.95***	
	1243.75	84.48 $\pm$ 3.52***		1300.00	87.12 $\pm$ 2.32***	
5	310.94	9.56 $\pm$ 3.68***	850.3	300.00	0.83 $\pm$ 0.63	— ^d
	621.88	52.12 $\pm$ 5.11***		600.00	1.67 $\pm$ 1.27	
	1243.75	73.28 $\pm$ 4.31***		1200.00	8.19 $\pm$ 1.65	
6	326.56	2.35 $\pm$ 3.49***	— ^c	323.44	3.34 $\pm$ 2.74	1210.8
	653.13	14.21 $\pm$ 5.77***		646.88	23.08 $\pm$ 4.00***	
	1306.25	29.83 $\pm$ 4.36***		1293.75	50.84 $\pm$ 5.78***	
Azoxystrobin	0.57	37.96 $\pm$ 2.41***	0.5	0.30	38.99 $\pm$ 3.54***	0.4
	1.15	66.78 $\pm$ 7.56***		0.60	69.13 $\pm$ 3.03***	
	2.29	81.48 $\pm$ 2.76***		1.20	88.53 $\pm$ 2.61***	

^a*n* = 6; ^bmean $\pm$ S.D.; ****P* < 0.001; ^cIC₅₀ > 1306.25 $\mu\text{g/mL}$ and not determined; ^dIC₅₀ > 1200.00 $\mu\text{g/mL}$ and not determined.

TABLE 6: Effect of gallic acid and methyl gallate on control of anthracnose disease of Chinese cabbage caused by *Colletotrichum higginsianum* PA-01 in growth chamber.

Treatment	Conc. ($\mu\text{g/mL}$)	5 days ¹		7 days		9 days	
		LN ²	LA (%)	LN	LA (%)	LN	LA (%)
CK	0	18.3 ^{a3}	40.6 ^a	27.3 ^a	64.4 ^a	34.8 ^a	81.6 ^a
Gallic acid	125	7.1 ^b	16.6 ^b	10.7 ^b	25.5 ^b	23.6 ^b	69.1 ^b
	250	4.4 ^{cd}	10.6 ^{cd}	9.6 ^{bc}	24.0 ^{bc}	20.1 ^{cd}	56.4 ^c
	500	4.0 ^{cd}	10.3 ^{cd}	8.6 ^{bcd}	23.3 ^{bc}	17.8 ^{de}	55.5 ^c
	1000	4.0 ^{cd}	8.7 ^{cd}	6.7 ^{cde}	19.7 ^{cd}	13.2 ^f	38.4 ^d
Methyl gallate	125	4.7 ^c	11.6 ^c	9.4 ^{bc}	21.0 ^{bcd}	21.3 ^{bc}	54.2 ^c
	250	4.6 ^c	11.3 ^{cd}	7.7 ^{bcd}	20.3 ^{cd}	19.0 ^{cd}	52.1 ^c
	500	3.4 ^d	7.9 ^d	6.0 ^{de}	17.9 ^d	14.9 ^{ef}	40.9 ^d
	1000	1.3 ^e	3.6 ^e	3.9 ^e	9.9 ^e	7.2 ^g	23.8 ^e
LSD _{0.05}		1.23	3.55	2.95	4.86	3.36	7.54

¹Days after inoculation. ²LN: lesion number per 3 cm in diameter of infected leaves and LA (%): percentage of lesion area per 3 cm in diameter of infected leaves. ³Data in each column with the same letter are significantly different according to LSD test in *P* = 0.05.

suppression by methyl gallate was better than by gallic acid in the same concentration (Figure 3). For example, the lesion number was not a significant difference in treatment of methyl gallate with 14.9 at 500 $\mu\text{g/mL}$ and in treatment of gallic acid with 13.2 at 1000 $\mu\text{g/mL}$ 9 days after inoculation. Similarity, lesion area (%) was also not a significant difference in treatment of methyl gallate with 40.9 at 500 $\mu\text{g/mL}$ and in treatment of gallic acid with 38.4 at 1000 $\mu\text{g/mL}$ 9 days after inoculation (Table 6). It means that lower concentration

of methyl gallate displayed better effects for disease control than higher concentration of gallic acid. The results would be valuable for the discovery of new plant-derived pesticide preparations.

4. Conclusion

The present investigation results indicate that the methanol extracts of *M. chinensis*, *E. caryophyllata*, *P. cuspidatum*,

FIGURE 2: Effect of methyl gallate on inhibition of conidial germination of *Colletotrichum higginsianum* PA-19. (a) An irregular brown appressorium (AP) formed after conidial germination in distilled water (check); and (b) conidium (C) failed to germinate in the solution with 22.1 $\mu\text{g/mL}$ of methyl gallate (bar scale = 20 μm).

FIGURE 3: Effect of gallic acid (1000 $\mu\text{g/mL}$) (A) and methyl gallate (1000 $\mu\text{g/mL}$) (B) on control of anthracnose disease of Chinese cabbage caused by *Colletotrichum higginsianum* PA-01.

and *R. officinale* may be potential for further development of plant-derived pesticides for control of anthracnose of cabbage and other cruciferous crops. The developed HPLC analytical methods are convenient and feasible tools for species authentication and quality assessment of the herbal raw materials. They are helpful to monitor the contents of active principles in the herbs for developing new botanical pesticides.

According to the experimental data in the present study, methyl gallate showed only 1/80 activity of the pesticide azoxystrobin; however, the herbal extracts would be safer and less dangerous to the ecosystem. These traditional Chinese medicines could be studied further for their cytotoxicity and synergistic effects of different combinations. It would be also potential to study the antifungal mechanism in the future.

Abbreviation List

HPLC: High performance liquid chromatography
 PDA: Potato dextrose agar
 LOD: Limit of detection

SD: Standard deviation

RSD: Relative standard deviation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors are grateful for the financial support from the Council of Agriculture, Executive Yuan, Taiwan, awarded to Dr. P. C. Kuo. This study is supported in part by grants awarded to Dr. T. F. Hsieh and Dr. P. C. Kuo from the Ministry of Science and Technology, Taiwan.

References

- [1] M. K. Kim, G. J. Choi, and H. S. Lee, "Fungicidal property of *Curcuma longa* L. rhizome-derived curcumin against phytopathogenic fungi in a greenhouse," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 6, pp. 1578–1581, 2003.
- [2] Y.-M. Kim, C.-H. Lee, H.-G. Kim, and H.-S. Lee, "Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 20, pp. 6096–6100, 2004.
- [3] A. Angioni, A. Barra, V. Coroneo, S. Dessi, and P. Cabras, "Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. *stoechas* essential oils from stem/leaves and Flowers," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 12, pp. 4364–4370, 2006.
- [4] W. C. Chung, J. W. Huang, H. C. Huang, and J. F. Jen, "Effect of ground *Brassica* seed meal on control of *Rhizoctonia* damping-off of cabbage," *Canadian Journal of Plant Pathology*, vol. 24, no. 2, pp. 211–218, 2002.
- [5] Y.-J. Ahn, H.-S. Lee, H.-S. Oh, H.-T. Kim, and Y.-H. Lee, "Antifungal activity and mode of action of *Galla rhois*-derived phenolics against phytopathogenic fungi," *Pesticide Biochemistry and Physiology*, vol. 81, no. 2, pp. 105–112, 2005.

- [6] W.-C. Ho, H.-J. Su, J.-W. Li, and W.-H. Ko, "Effect of extracts of Chinese medicinal herbs on spore germination of *Alternaria brassicicola*, and nature of an inhibitory substance from gallnuts of Chinese sumac (*Rhus chinensis*)," *Canadian Journal of Plant Pathology*, vol. 28, no. 4, pp. 519–525, 2006.
- [7] D. Jasso De Rodríguez, D. Hernández-Castillo, R. Rodríguez-García, and J. L. Angulo-Sánchez, "Antifungal activity in vitro of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi," *Industrial Crops and Products*, vol. 21, no. 1, pp. 81–87, 2005.
- [8] V.-N. Nguyen, D.-M. Nguyen, D.-J. Seo, R.-D. Park, and W.-J. Jung, "Antimycotic activities of cinnamon-derived compounds against *Rhizoctonia solani* in vitro," *BioControl*, vol. 54, no. 5, pp. 697–707, 2009.
- [9] A. Cakir, S. Kordali, H. Kilic, and E. Kaya, "Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse," *Biochemical Systematics and Ecology*, vol. 33, no. 3, pp. 245–256, 2005.
- [10] A. Barra, V. Coroneo, S. Dessi, P. Cabras, and A. Angioni, "Characterization of the volatile constituents in the essential oil of *Pistacia lentiscus* L. from different origins and its antifungal and antioxidant activity," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 17, pp. 7093–7098, 2007.
- [11] V. K. Bajpai, A. Rahman, and S. C. Kang, "Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu," *Industrial Crops and Products*, vol. 26, no. 1, pp. 28–35, 2007.
- [12] V. K. Bajpai, S. Shukla, and S. C. Kang, "Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L," *Bioresource Technology*, vol. 99, no. 18, pp. 8903–8908, 2008.
- [13] C. L. Wilson, J. M. Solar, A. El Ghaouth, and M. E. Wisniewski, "Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*," *Plant Disease*, vol. 81, no. 2, pp. 204–210, 1997.
- [14] Y. L. Chu, W. C. Ho, and W. H. Ko, "Effect of Chinese herb extracts on spore germination of *Oidium murrayae* and nature of inhibitory substance from Chinese rhubarb," *Plant Disease*, vol. 90, no. 7, pp. 858–861, 2006.
- [15] C. L. Lin, T. C. Lin, and J. W. Huang, "Evaluation for efficacy of clove oil and plant nutrients on controlling the cruciferous vegetable anthracnose caused by *Colletotrichum higginsianum*," *Plant Pathology Bulletin*, vol. 19, pp. 167–176, 2010.
- [16] J. A. Bailey and M. J. Jeger, *Colletotrichum: Biology, Pathology and Control*, CAB International, Wallingford, UK, 1992.
- [17] R. D. Fitzell and C. M. Peak, "The epidemiology of anthracnose disease of mango: inoculum sources, spore production and dispersal," *Annals of Applied Biology*, vol. 104, pp. 53–59, 1984.
- [18] S. Freeman, T. Katan, and E. Shabi, "Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits," *Plant Disease*, vol. 82, no. 6, pp. 596–605, 1998.
- [19] R. Thangavelu, P. Sundararaju, and S. Sathiamoorthy, "Management of anthracnose disease of banana caused by *Colletotrichum musae* using plant extracts," *Journal of Horticultural Science and Biotechnology*, vol. 79, no. 4, pp. 664–668, 2004.
- [20] R. O'Connell, C. Herbert, S. Sreenivasaprasad, M. Khatib, M.-T. Esquerré-Tugayé, and B. Dumas, "A novel *Arabidopsis-Colletotrichum* pathosystem for the molecular dissection of plant-fungal interactions," *Molecular Plant-Microbe Interactions*, vol. 17, no. 3, pp. 272–282, 2004.
- [21] Y. Narusaka, M. Narusaka, P. Park et al., "RCH1, a locus in *Arabidopsis* that confers resistance to the hemibiotrophic fungal pathogen *Colletotrichum higginsianum*," *Molecular Plant-Microbe Interactions*, vol. 17, no. 7, pp. 749–762, 2004.
- [22] A. Huser, H. Takahara, W. Schmalenbach, and R. O'Connell, "Discovery of pathogenicity genes in the crucifer anthracnose fungus *Colletotrichum higginsianum*, using random insertional mutagenesis," *Molecular Plant-Microbe Interactions*, vol. 22, no. 2, pp. 143–156, 2009.
- [23] C. L. Lin and J. W. Huang, "The occurrence of cruciferous vegetable anthracnose in Taiwan and identification of its pathogen," *Plant Pathology Bulletin*, vol. 11, pp. 173–178, 2002.
- [24] P.-C. Kuo, T.-C. Lin, C.-W. Yang, C.-L. Lin, G.-F. Chen, and J.-W. Huang, "Bioactive saponin from tea seed pomace with inhibitory effects against *Rhizoctonia solani*," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 15, pp. 8618–8622, 2010.
- [25] Y. H. Lee and R. A. Dean, "cAMP regulates infection structure formation in the plant pathogenic fungus *Magnaporthe grisea*," *Plant Cell*, vol. 5, no. 6, pp. 693–700, 1993.
- [26] J. J. Lu, Y. Wei, and Q. P. Yuan, "Preparative separation of gallic acid from Chinese traditional medicine by high-speed counter-current chromatography and followed by preparative liquid chromatography," *Separation and Purification Technology*, vol. 55, no. 1, pp. 40–43, 2007.
- [27] Z.-D. He, C.-F. Qiao, Q.-B. Han et al., "Authentication and quantitative analysis on the chemical profile of Cassia Bark (*Cortex cinnamomi*) by high-pressure liquid chromatography," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 7, pp. 2424–2428, 2005.
- [28] G. Qian, S.-Y. Leung, L. U. Guanghua, and K. S.-Y. Leung, "Differentiation of *Rhizoma et Radix Polygoni Cuspidati* from closely related herbs by HPLC fingerprinting," *Chemical and Pharmaceutical Bulletin*, vol. 54, no. 8, pp. 1179–1186, 2006.
- [29] P. C. Kuo and Y. L. Chang, "Fingerprint chromatogram analysis of methanol extracts of *Gardenia jasminoides* and *Arctium lappa* by high performance liquid chromatography," *Analytical Chemistry*, vol. 11, pp. 162–168, 2012.
- [30] K. J. Brent, D. W. Hollomon, and M. W. Shaw, "Predicting the evolution of fungicide resistance," in *Managing Resistance to Agrochemicals*, M. B. Green, H. M. LeBaron, and W. K. Moberg, Eds., pp. 303–319, American Chemical Society, Washington, DC, USA, 1990.
- [31] H. C. Huang and C. H. Chou, "Impact of plant disease biocontrol and allelopathy on biodiversity and agricultural sustainability," *Plant Pathology Bulletin*, vol. 14, no. 1, pp. 1–12, 2005.
- [32] M. Muto, H. Takahashi, K. Ishihara, H. Yuasa, and J. W. Huang, "Control of black leaf spot (*Alternaria brassicicola*) of crucifers by extracts of black nightshade (*Solanum nigrum*)," *Plant Pathology Bulletin*, vol. 14, pp. 25–36, 2005.
- [33] J. H. Yen, D. Y. Chen, W. C. Chung, T. T. Tsay, and T. F. Hsieh, "Effect of natural plant protectants on controlling plant-parasitic nematode disease," *Plant Pathology Bulletin*, vol. 17, pp. 169–176, 2008.
- [34] J. T. Li, B. L. Yin, Y. Liu, L. Q. Wang, and Y. G. Chen, "Monoaromatic constituents of *Dendrobium longicornu*," *Chemistry of Natural Compounds*, vol. 45, pp. 234–236, 2009.
- [35] S. E. Choi, J. H. Yoon, H. K. Choi, and M. W. Lee, "Phenolic compounds from the root of *Phragmites communis*," *Chemistry of Natural Compounds*, vol. 45, no. 6, pp. 893–895, 2009.
- [36] C. Y. Chen, H. M. Wang, S. H. Chung, W. L. Lo, W. L. Yang, and S. C. Yang, "Chemical constituents from the roots of

- Cinnamomum subavenium*,” *Chemistry of Natural Compounds*, vol. 46, no. 3, pp. 474–477, 2010.
- [37] J. Savard and P. Brassard, “Reactions of ketene acetals-14. The use of simple mixed vinylketene acetals in the annulation of quinones,” *Tetrahedron*, vol. 40, no. 18, pp. 3455–3464, 1984.
- [38] M. R. Meselhy, “Constituents from Moghat, the roots of *Glossostemon bruguieri* (Desf.),” *Molecules*, vol. 8, no. 8, pp. 614–621, 2003.
- [39] T. Sassa, “Overproduction of moniliphenone , a benzophenone biosynthetic intermediate of chloromonilicin , by *Monilinia fructicola* and its anthraquinone precursors,” *Agricultural and Biological Chemistry*, vol. 55, no. 1, pp. 95–99, 1991.