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Antimicrobial Activity of Medicinal Plants Used by Indigenous People in Taiwan

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ABSTRACT

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Extracts derived from fresh and dry tissues of 14 plant species used in ethnobotanical medicine by the indigenous Tsou people in Taiwan were evaluated for activities against 10 species of plant pathogens, including seven fungi: *Alternaria brassicicola*, *Colletotrichum higginsianum*, *Cylindrocladium scoparium*, *Fusarium moniliforme*, *Phytophthora infestans*, *Pythium aphanidermatum*, and *Rhizoctonia solani* and three bacteria: *Erwinia carotovora* subsp. *carotovora*, *Pseudomonas syringae*, and *Xanthomonas campestris* pv. *vesicatoria*. Results showed that inhibition of spore germination, mycelial growth, and cell proliferation of the plant pathogens varied with plant extracts, microorganisms and extraction methods. Hot water extracts of fresh tissues of *Solanum nigrum* (SN) at 20-fold dilutions completely inhibited spore germination of *A. brassicicola*. Ethanol extracts from fresh and dry tissues of SN inhibited spore germination of *A. brassicicola* at the concentrations of 160-fold and 400-fold dilutions, respectively. In inoculation study, the severity of black leaf spot of crucifer caused by *A. brassicicola* was significantly reduced to below 27% for the treatments of extracts from 10% (w/v) of fresh and 1% (w/v) of dry tissues of SN, compared to the severity of 97.2% in water and ethanol controls. The water extract from 10% (w/v) of fresh *Pueraria montana* tissues was the most effective treatment against anthracnose of crucifer caused by *C. higginsianum* and the disease severity was 22.2%, compared to 94.4% for the untreated controls. Although both ethanol and water extracts from fresh tissues of *Clematis tashiroi* completely suppressed the lesion development of tomato late blight caused by *P. infestans* at a concentration of 10% (w/v), same suppressive effects at lower concentrations were observed only in the treatments of 2.5% (w/v) water extract from fresh tissues and 0.5% (w/v) water extract from dry tissues. These results suggest the possibility of control of plant diseases by natural substances from some ethnobotanical medicinal plants used by the indigenous people in Taiwan.

Key words : Biocontrol, botanical pesticide, ethnobotanical medicinal plant, plant extract

INTRODUCTION

Pest management is indispensable for sustaining economically viable agricultural production [11, 13, 16, 27]. During the past few decades, synthetic pesticides have been used heavily in agriculture in order to control crop pests and improve crop yields [14, 15]. However, the use of highly toxic synthetic pesticides in crop production is harmful to the environme the ecosystems and human and animal health. Thus, there i demand for environmentally-sound methods as alternatives synthetic pesticides for control of crop pests. T development of natural products for pest control presents ideal method for sustainable agricultural productions of cr with minimum detrimental effects to the environment [35].

Numerous diverse chemical compounds are produced by higher plants and each compound exhibits specific biological functions [10, 11, 13, 16, 33, 36]. Previous reports indicate that certain plant species contain specific compounds that are of pesticide values. For example, extracts from neem tree [2, 17, 27, 31], camphor tree [10, 27], clove [12, 27], pyrethrum [11, 27], and *Brassica* spp. [4, 9, 22, 23, 24] have been shown to have potent pesticidal properties. Therefore, these plant products and their related chemical analogues may implicate a potential source of natural plant substances for control of insect pests and plant pathogens. Other reports suggest that certain plant species used in traditional human medicine may also constitute effective compounds for control of crop diseases [21, 26, 29, 34].

A large number of plant species grown in Taiwan is reported to have medicinal properties [7, 8]. Historically, many of these plants have been commonly used for ethnobotanical medicine by indigenous Formosan people. There are nine ethnic groups of indigenous people in Taiwan, including Tsou, Amis, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, and Yami (Tao). Each ethnic group maintained their distinct language, culture, and lifestyle, and inhabited in a specific region of the Taiwan island [37]. For example, the Tsou people inhabited in the mountainous areas of south central Taiwan. They used numerous species of native plants grown in south central region of Taiwan as ethnobotanical medicine [6]. Whether these medicinal plants contain antifungal or antibacterial properties remain unknown.

The objectives of this study were to evaluate antimicrobial activities of aqueous and ethanol extracts of ethnobotanical medicinal plants collected from mountainous areas of south central Taiwan and determine their potential for control of plant diseases.

MATERIALS AND METHODS

Plant materials

Fourteen species of medicinal plants were used in this study (Table 1). They were collected from Tappang, Chia-yi County, Taiwan. The taxonomy of these plant materials was confirmed by Drs. S. C. Liu and J. L. Tsai, Department of Life Science, National Chung Hsing University (NCHU), Taichung, Taiwan. Plant species and plant parts (Table 1) used in the experiments were selected based on the information of their medicinal properties obtained through verbal interviews with the indigenous Tsou people in Taiwan.

Preparation of plant extracts

Water and ethanol extracts were prepared from fresh and air-dried samples of each plant species using a modified method described previously [2, 28, 30]. Two water extracts were prepared from each plant sample; one was cool water (25°C) and the other was hot water (at 80°C). For the extract at 25°C, the fresh plant materials were first washed in running tap water followed by washing in distilled water, air-dried, and cut into pieces of approximately 2-3 cm in length. The tissues were homogenized in distilled water (1:5; w/v) using a blender. After 24 h, the mixture was filtered through double-layered cheesecloth and the filtrate was centrifuged (Sigma, MO, USA) at 2000 g for 5 minutes. The supernatant was passed through a No. 1 filter paper (Toyo Roshi Co., Japan) and then through a 0.45 μm polyvinylidene difluoride (PVDF) membrane filter (Millipore Co., Bedlford, MA, USA) to collect the water extract. For water extracts from air-dried samples, 10 ml of distilled water (25°C) were added to two grams of dried plant powders, agitated for 24 h on an orbital shaker (80 rpm; Firstek Scientific Model S102; Hsin Chuang,

Table 1. List of 14 species of ethnobotanical medicinal plants collected from Tappang, Chia-yi county, Taiwan, and used in the study

Plant species	Abbreviation	Family	Type of tissues	Function[1]
Alpinia speciosa	AS	Zingiberaceae	Root	Swelling, injury
Arenga engleri	AE	Palmae	Aboveground tissues	Headache, stomachache
Blumea lanceolaria	BL	Compositae	Whole plant	Ophthalmia
Clematis tashiroi	CT	Ranunculaceae	Root	Detoxification (snake)
Drymaria diandra	DD	Caryophyllaceae	Whole plant	Dermatitis
Gonostegia hirta	GH	Urticaceae	Root	Swelling
Houttuynia cordata	HC	Saururaceae	Root	Vermifuge
Plantago asiatica	PA	Plantaginaceae	Whole plant	Cough remedy
Pueraria montana	PM	Leguminosae	Young shoot	Styptic
Rubia akane	RA	Rubiaceae	Root	Bronchitis
Rumex japonicus	RJ	Polygonaceae	Leaf	Antipruritic
Sambucus chinensis	SC	Caprifoliaceae	Leaf	Fatigue
Solanum nigrum	SN	Solanaceae	Root	Bronchitis, stomachache
Uraria crinita	UC	Leguminosae	Root	Stomachache, diarrhea

[1] Personal communication with Tsou, indigenous Formosan people.

Taiwan, ROC) and filtered to collect water extracts by the same procedure described above.

For hot water extracts, fresh plant materials were mixed with distilled water (1:5; w/v) and homogenized in a blender. The mixtures were poured into flasks, placed in a hot water bath (80°C) on a shaker at 80 rpm for 30 min and water extracts were collected by the same procedure described above. Same procedures were used for hot water extraction of dry plant tissues except the tissue: water ratio was 1:50 (w/v).

For preparation of plant extracts in ethanol, 20 g of small pieces of fresh plant tissues were added to 37.5 ml of 80% ethanol and homogenized in a blender. The blended tissues in ethanol were kept at room temperature (25°C) for 24 h and then diluted to the final ethanol concentration of 30% using 62.5ml of distilled water and filtered through double-layered cheesecloth to collect extracts. Same ethanol extraction procedures were used for dry plant tissues except the final concentration of 2% (w/v) materials in 30% (v/v) ethanol.

Preparation of plant pathogens

Ten plant pathogens, including seven fungi from the Laboratory of Plant Disease Management, NCHU, Taiwan and the Plant Pathology Division, Taiwan Agricultural Research Institute and three bacteria from the Laboratory of Bacterial Diseases, NCHU, were used in this study (Table 2). Stock cultures of the fungal pathogens were maintained on rye B medium (RB)[5] and potato dextrose agar (PDA) (Difco, Detroit, MI, USA) at room temperature (22-25°C) under 12 h diurnal illumination.

The working cultures of *Phytophthora infestans* (Mont.) de Bary (isolate PI-109) were grown on RB medium in Petri dishes at 20°C in dark for 10-14 days. Sporangia were harvested by flooding the cultures with sterile distilled water and filtering the suspension through a double-layered cheesecloth to remove mycelial fragments. The concentration of sporangia in the filtrate was adjusted to 2×10^4 sporangia/ml. To obtain zoospores, the sporangial suspension of *P. infestans* v inoculated on detached tomato leaves [32]. The inocula leaves were placed in a sterilized Petri dish at high humic (RH > 70%) and incubated at 16°C overnight then at 20°C 7 days. The infected leaves were rinsed with sterile distil water and agitated gently for several minutes. The sporan suspension was chilled at 4°C for 2 h to induce the release zoospores, and then placed at room temperature immediate preparation of zoospore suspensions for this stu

Working cultures of *Alternaria brassicicola* (Sch Wiltshire (isolate ABA-31), *Colletotrichum higginsian* Sacc. (isolate PA-01), *Cylindrocladium scoparium* Morg (isolate CS-01), and *Fusarium moniliforme* Sheldon (isol FM-024) were prepared from 14-day-old, PDA slant cultu Conidial suspensions were made by adding 10 ml ste distilled water to each tube, rubbing the surface of the col with a scalpel to dislodge spores and filtering the solut through double-layered cheesecloth to collect the sp suspension. The concentration of conidia in each suspens was adjusted using microsyringe method [20]. For work cultures of *Pythium aphanidermatum* (Edson) Fitzpatr (isolate PAM-2) and *Rhizoctonia solani* Kühn AG2-2I (isolate R-505), agar disks containing mycelial mats remo from 3-day-old, PDA cultures in Petri dishes were used inoculum.

For the three bacterial pathogens, *Erwinia carotov* subsp. *carotovora* Bergyetal (strain GL1) and *Xanthomo campestris* pv. *vesicatoria* (Doidge) Dowson (XVT40) w cultured on nutrient agar (NA) (Difco, Detroit, MI, USA) a *Pseudomonas syringae* van Hall (PA5) was grown on Kin B medium (KB) [19]. The cultures were incubated at 30°C 2-3 days, and then used for preparation of bacter suspensions containing 1×10^8 cfu/ml for the experiments.

Assay of antimicrobial activities in plant extracts

The *in vitro* antimicrobial activity of the plant extra

Table 2. List of fungal and bacterial plant pathogens used in this study

Plant pathogen	Strain	Host plant	Source [1]
Fungi			
Alternaria brassicicola	ABA-31	Chinese cabbage	1
Colletotrichum higginsianum	PA-01	Chinese cabbage	1
Cylindrocladium scoparium	CS-01	Rose	1
Fusarium moniliforme	FM-024	Rice	1
Phytophthora infestans	PI-109	Potato	1
Pythium aphanidermatum	PAM-2	Cucumber	1
Rhizoctonia solani AG2-2ⅢB	R-505	Amaranths	3
Bacteria			
Erwinia carotovora subsp. *carotovora*	GL1	Color lily	2
Pseudomonas syringae	PA5	Star fruit	2
Xanthomonas campestris pv. *vesicatoria*	XVT40	Sweet pepper	2

[1] Source of plant pathogens: 1, from the Laboratory of Plant Disease Management, National Chung Hsing University (NCHU), Taiwan from the Laboratory of Plant Bacterial Diseases, NCHU, Tawan; and 3, from the Agricultural Research Institute, Taichung, Taiwan.

was determined by the spore germination or mycelial growth assay for the fungi and the disk diffusion assay for growth of the bacteria. Plant extracts showing inhibitory effects on the organisms were selected for further testing. The minimum inhibitory concentration (MIC) value was defined as the lowest extract concentration required for complete suppression of growth of a tested fungus [3].

1. Spore germination assay

Extracts from fresh and dry tissues of 14 species of plants were tested for inhibition of spore germination of five fungal plant pathogens, including *A. brassicicola*, *C. higginsianum*, *C. scoparium*, *F. moniliforme*, and *P. infestans*. It was conducted by placing four drops (10 μl/drop) of spore suspension (2×10^4 spore/ml) of each pathogen on a glass slide. Ten μl of cool water extract, hot water extract, or ethanol extract from 2% (w/v) of dry plant tissues or 20% (w/v) of fresh plant tissues was added to each drop of the spore suspension. The same volume of sterile distilled water or 30% (v/v) ethanol was added to each drop of the spore suspension as controls. The glass slides were placed on a moist filter paper in Petri dishes, 1 slide/dish, and incubated at 24℃ for 12 h for *A. brassicicola* and for 24 h for *C. higginsianum*; at 28℃ for 6 h for *C. scoparium* and for 4 h for *F. moniliforme*; and at 20℃ for 3 h for *P. infestans*. After incubations, the cultures on each slide were stained with lactophenol cotton blue and examined microscopically to determine germination of 100 randomly selected spores in each tested sample. Each experiment was repeated three times with four replicates per treatment in each experiment.

2. Effects of plant extracts on mycelial growth of fungi

The effects of extracts from medicinal plants on mycelial growth of *P. aphanidermatum* and *R. solani* were determined using 2% (w/v) water agar (WA) (Difco, Detroit, MI, USA). For each WA dish (9-cm diam.), 200 μl of each extract were flooded on the agar surface to form a thin film. For controls, each agar dish was flooded either with 200 μl of sterile distilled water or with 15% (v/v) ethanol. An agar disk (4-mm diam.) containing mycelial mats, was removed from 3-day-old cultures of each fungus, and placed on the medium at the center of the Petri dish. The dishes were incubated at 28℃ for 12 h for *P. aphanidermatum* and at 24℃ for 24 h for *R. solani* and the colony diameter in each dish was measured. To determine inhibitory effects of plant extracts, the differences of colony diameters between each treatment of plant extract and untreated controls were calculated according to the formula of Pandey *et al*. (1982) [25]:

$$\text{Inhibition (\%)} = \frac{\text{colony diameter in control-colony diameter in treated culture}}{\text{colony diameter in control}} \times 100$$

For each experiment, there were four replicates (Petri dishes) in each treatment and the experiment was repeated twice.

Effects of plant extracts on growth of bacteria

One ml of each bacterial suspension was flooded on the surface of nutrient agar (NA) in each Petri dish. Filter paper disks (8 mm in diam.) (Toyo Roshi Co., Japan) were immersed in each plant extract which was diluted with sterile distilled water at 1: 1 ratio (v/v) and four disks were placed on each bacterium-inoculated agar dish. Filter paper disks soaked in sterile distilled water or 15% (v/v) ethanol were used as controls. To determine the antibacterial activity of each plant extract, the dishes were incubated at 30℃ for 48 h, and then compared the difference in zone of growth inhibition between treated and untreated controls. This experiment was repeated twice with four replicates per treatment for each experiment.

Effect of plant extracts on control of fungal plant diseases

Effects of plant extracts on suppression of fungal plant diseases were assessed using the detach-leaf method. The leaves of Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) cv. Known-You No. 36 (Known-You Seed Co., Taiwan) were used for *A. brassicicola* and *C. higginsianum*, and the leaves of tomato (*Lycopersicum esculentum* Mill.) cv. Known-You No. 301 were used for *P. infestans*. For Chinese cabbage, the 3rd to 5th true leaves were removed, washed briefly in distilled water, immersed in 70% (v/v) ethanol for 30 sec, rinsed with sterile distilled water and placed on sterile moist filter paper in Petri dishes (RH > 70%), two leaves per dish. For each treatment, 10 μl of spore suspension (2×10^5 spores per ml) of the pathogen (*A. brassicicola* or *C. higginsianum*) were applied to the surface of each leaf. Controls were set up by inoculation of each leaf with 10 μl of sterile distilled water or 30% (v/v) ethanol. After incubation at 24℃ for 3 days, the disease severity of *A. brassicicola* and *C. higginsianum* on Chinese cabbage was evaluated by the formula of James (1971) [18]:

$$\text{Disease severity (\%)} = \frac{0\times n_0+1\times n_1+2\times n_2+3\times n_3+4\times n_4}{N\times 4}\times 100,$$

where n_0 = number of healthy leaves per plant, n_1 = number of leaves showing 1-10% leaf area infected, n_2 = number of leaves showing 11-25% leaf area infected, n_3 = number of leaves showing 26-50% leaf area infected, n_4 = number of leaves showing >50% leaf area infected, and N = total number of leaves in each replicate.

The same inoculation procedure described above was used for *P. infestans* on tomato with the modifications that the leaves were harvested from 2nd to 4th leaves of 7-wk-old tomato plants and disease assessment was done at 7 days after inoculation. The incidence of tomato late blight caused by *P. infestans* was recorded as positive (+) or negative (-)where positive represents leaf with lesions and negative represents leaf without lesions. There were three replications for each treatment and the experiment was repeated twice.

Table 3. Effects of aqueous or ethanol extracts of 14 species of medicinal plants on spore germination of five fungal plant pathoge

Plant[1]	Type of tissues[2]	Spore germination (%)[3]													
		ABA-31[1]			PA-01			CS-01			FM-024			PI-109	
		W[4]	H	Et	W	H	Et	W	H	Et	W	H	Et	W	H
AS	Fresh	4	4	4	0	4	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	3	4	4	1	4	3
AE	Fresh	4	4	4	0	4	4	4	4	4	4	4	4	2	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	4	4
BL	Fresh	4	4	4	0	0	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	4	4
CT	Fresh	4	4	4	0	4	4	4	4	1	4	4	0	0	0
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	0	3
DD	Fresh	4	4	4	0	4	4	4	4	4	4	0	1	0	0
	Dry	4	4	4	4	4	4	4	4	4	4	4	3	3	4
GH	Fresh	4	4	4	0	4	4	4	4	4	4	4	2	4	2
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	3	3
HC	Fresh	4	4	4	0	0	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	4	3
PA	Fresh	4	4	4	0	4	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	3	3
PM	Fresh	4	4	4	0	0	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	4	4
RA	Fresh	4	4	4	4	1	0	4	4	1	4	4	4	2	3
	Dry	4	4	4	0	1	1	4	4	2	4	4	4	3	3
RJ	Fresh	4	4	4	4	2	4	4	4	4	4	4	4	4	2
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	3	3
SC	Fresh	4	4	4	0	4	4	4	4	4	4	4	4	3	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	2	4	3
SN	Fresh	0	0	0	0	4	4	4	4	4	4	4	4	3	3
	Dry	0	0	0	0	4	4	4	4	4	4	4	4	3	3
UC	Fresh	4	4	4	3	0	4	4	4	4	4	4	4	4	4
	Dry	4	4	4	0	4	4	4	4	4	4	4	4	4	3
CK	Water	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	EtOH	nd	nd	4	nd	nd	4	nd	nd	4	nd	nd	4	nd	nd

[1] Abbreviations of plant species and plant pathogens were listed in Table 1 and Table 2, respectively.

[2] Fresh or dry materials were used to prepare cool water (25°C), hot water (80°C), or ethanol extracts. Sterile distilled water and 15% (v ethanol were used as untreated controls (CK).

[3] Spore germination was recorded based on random checking of 100 conidia in each sample. All spore germination data were means three experiments, four replicates/treatment. They were designated by the score of 0 to 4: 0, no germination; 1= 1-10% of spo germinated; 2= 11-25% of spores germinated; 3= 26-50% of spores germinated; 4= >50% of spores germinated; and nd= no data.

[4] Solvents for extract preparation: W= Distilled water (25°C), H= Hot water (80°C), and Et= 80% (v/v) ethanol (final concentration v adjusted to 15% (v/v)).

Statistical analyses

Data from the inoculation trials using detached leaves were evaluated by analysis of variance (ANOVA) with the SAS statistical software (SAS Institute, Inc., Cary, NC). Means of the treatments in each experiment were compared by Tukey's studentized range test at $P = 5\%$ level.

RESULTS

Antimicrobial activity of medicinal plant extracts

Inhibitory effects of plant extracts from 14 species medicinal plants on spore germination of five fungal pl pathogens varied with plant species, plant pathogens a methods of extractions (Table 3). For example, amo extracts of fresh and dry tissues of 14 species of plants test only *Solanum nigrum* (SN) prepared in cool water (25°C), water (80°C) or ethanol (15%) showed complete suppressior spore germination of *A. brassicicola* (ABA-31) but they w

ineffective in suppression of spore germination of other fungi such as *C. scoparium* (PA-01), *F. moniliforme* (FM-024), and *P. infestans* (PI-109). A complete suppression of spore germination of *C. higginsianum* by SN was also obtained but only by the cool water extraction (Table 3). Results also showed that extracts from all 14 species of plants were effective in suppression of spore germination of *C. higginsianum* and the extracts prepared by the cool water (25℃) extraction were generally more effective than those prepared by hot water (80 ℃) and ethanol extractions (Table 3). In addition, spores of *P. infestans* treated with extracts from fresh or dry tissues of *Clematis tashiroi* (CT) prepared in cool water (28℃) also failed to germinate. No inhibitory effects on germination of spores were observed for the water or ethanol (15%) controls in the five plant pathogenic fungi tested.

The effects of plant extracts from 14 species of medicinal plants on mycelial growth of *P. aphanidermatum* (PAM-02) and *R. solani* (R-505) varied with plant species, plant pathogens and methods of extractions (Table 4). Of the 14 plant species tested, only the extracts from fresh tissues of *C. tashiroi* (CT) and *Drymaria diandra* (DD) showed a complete inhibition of mycelial growth of *P. aphanidermatum*

Table 4. Effects of aqueous or ethanol extracts of 14 species of medicinal plants on mycelial growth of two fungal plant pathogens

Plant[1]	Type of tissues[2]	Inhibition of mycelial growth[3]					
		PAM-02[1]			R-505		
		Water[4]	Hot water	Ethanol	Water	Hot water	Ethanol
AS	Fresh	+	—	—	+	—	—
	Dry	+	+	—	+	+	—
AE	Fresh	+	—	—	+	—	—
	Dry	++	—	—	+	—	—
BL	Fresh	—	+	—	+	—	—
	Dry	+	—	—	+	—	—
CT	Fresh	++++	+	++++	++++	++++	++++
	Dry	+	+	—	+	++	—
DD	Fresh	—	++++	—	+	++++	—
	Dry	+	—	—	+	++	—
GH	Fresh	—	—	—	+	—	—
	Dry	+	—	—	+	+	—
HC	Fresh	—	—	—	+	—	—
	Dry	+	—	—	+	—	—
PA	Fresh	+	—	—	+	—	—
	Dry	+	—	—	+	+	—
PM	Fresh	—	—	—	+	—	—
	Dry	+	—	—	—	+	—
RA	Fresh	—	—	—	+	—	++
	Dry	+	—	—	+	—	—
RJ	Fresh	—	—	—	—	—	+
	Dry	+	—	—	—	+	—
SC	Fresh	+	—	—	+	—	—
	Dry	+	—	—	+	+	—
SN	Fresh	—	—	—	+	+	—
	Dry	+	—	—	+	—	+
UC	Fresh	—	—	—	+	+	—
	Dry	+	—	—	+	+	—
CK	Water	—	nd	nd	—	nd	nd
	EtOH	nd	nd	—	nd	nd	—

[1] Abbreviations of plant species and plant pathogens were listed in Table 1 and Table 2, respectively.

[2] Fresh or dry materials were used to prepare cool water (25℃), hot water (80 ℃), or ethanol extracts. Sterile distilled water and 15% (v/v) ethanol were used as untreated controls (CK).

[3] Inhibition of mycelial growth (%) = (colony size in control − colony size in treatment)/ colony size in control × 100. All data were means from four replicates and repeated twice. The data were designated as Inhibition index: -, no inhibition; +, 1-49% inhibition; ++, 50-74% inhibition; +++, 75-99% inhibition; ++++, 100% inhibition; and nd, no data.

[4] Solvents for extract preparation: were distilled water (25℃), hot water (80℃), and 80% (v/v) ethanol (final concentration was adjusted to 15% (v/v)).

and *R. solani*. Moreover, only the hot water extract from fresh tissues of *D. diandra* caused a complete inhibition of mycelial growth of these two pathogens, whereas the cool warter and ethanol extracts from fresh tissues of *C. tashiroi* were both effective in causing complete inhibition of mycelial growth of these pathogens (Table 4). Other preparations such as the water extract from dry tissues of *Arenga engleri* (AE) was effective in reducing more than 50% of mycelial growth of *P. aphanidermatum* and the ethanol extract from fresh tissues of *Rubia akane* (RA) was effective in reducing more than 50% reduction of mycelial growth of *R. solani*. No inhibition of mycelial growth was observed in the water and ethanol controls for both plant pathogens.

The extracts of the 14 plant species were not inhibit to the growth of the bacterial plant pathogens *E. carotov* subsp. *carotovora* (GL1) and *X. campestris* pv. *vesicato* (XVT40), regardless the extraction methods and the type tissues used (Table 5). However, extracts from 11 of the plant species showed inhibitory effects on growth of *syringae* (PA5). Strong inhibition of bacterial growth with inbition zone greater than 6 mm were observed in t treatments of extracts from plants species *C. tashiroi*, *diandra*, *Gonostegia hirta* (GH) or *Rumex japonicus* ((Table 5). Moreover, most of the inhibitory substances w present in the cool water extracts but absent in the etha extracts. In most instances, the antibacterial activity in

Table 5. Effects of aqueous or ethanol extracts of 14 species of medicinal plants on growth of three bacterial plant pathogens

Plant[1]	Type of tissues[2]	Inhibition zone (mm)[3]								
		GL1[1]			PA5			XVT40		
		W[4]	H	Et	W	H	Et	W	H	E
AS	Fresh	0.0	0.0	0.0	2.7±0.9	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	4.6±1.2	0.0	0.0	0.0	0.0	0.
AE	Fresh	0.0	0.0	0.0	4.0±0.5	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	1.6±0.4	0.0	0.0	0.0	0.0	0.
BL	Fresh	0.0	0.0	0.0	5.4±1.0	3.7±1.1	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
CT	Fresh	0.0	0.0	0.0	5.0±0.9	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	6.5±2.2	0.0	0.0	0.0	0.0	0.
DD	Fresh	0.0	0.0	0.0	7.2±1.2	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	5.9±2.7	0.0	0.0	0.0	0.0	0.
GH	Fresh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	8.6±4.3	4.8±0.9	0.0	0.0	0.0	0.
HC	Fresh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
PA	Fresh	0.0	0.0	0.0	0.0	5.3±0.7	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	4.1±1.7	0.0	0.0	0.0	0.0	0.
PM	Fresh	0.0	0.0	0.0	4.7±1.0	4.3±1.8	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	4.3±0.4	0.0	0.0	0.0	0.0	0.
RA	Fresh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
RJ	Fresh	0.0	0.0	0.0	0.0	6.2±1.2	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	6.6±1.1	0.0	0.0	0.0	0.0	0.
SC	Fresh	0.0	0.0	0.0	3.6±0.6	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	3.3±0.7	0.0	0.0	0.0	0.0	0.
SN	Fresh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
UC	Fresh	0.0	0.0	0.0	4.3±1.3	3.3±1.0	0.0	0.0	0.0	0.
	Dry	0.0	0.0	0.0	4.4±0.8	0.0	0.0	0.0	0.0	0.
CK	Water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.
	EtOH	nd	nd	0.0	nd	nd	0.0	nd	nd	0.

[1] Abbreviations of the plant species and plant pathogens were listed in Table 1 and Table 2, respectively.

[2] Fresh or dry materials were used to prepare cool water (25℃), hot water (80℃), or ethanol extracts. Sterile distilled water and 15% (v ethanol were used as untreated controls (CK).

[3] Values are means of four paper discs in each treatment with three replicates ± SD. nd: no data.

[4] Solvents for extract preparation: W= Distilled water (25℃), H= Hot water (80℃), and Et= 80% (v/v) ethanol (final concentration v adjusted to 15% (v/v)).

samples of cool water extracts was greater than the samples of hot water extracts for the plant species showing toxic effects on *P. syringae* (Table 5).

Minimum inhibitory concentration (MIC) of plant extracts with antifungal activities

The minimum inhibitory concentration (MIC) was determined for 39 of 84 plant extracts which completely inhibited mycelial growth and spore germination of fungi at a concentration of 10% (w/v) from fresh tissues and 1% (w/v) from dry tissues. Three fungi, *A. brassicicola*, *C. higginsianum*, and *P. infestans*, were used to determine the MIC values of the extracts from five plant species (Table 6). Extracts from fresh tissues of *S. nigrum* exhibited antifungal activity against *A. brassicicola* at the low MIC (160-fold dilution for ethanol extracts). In other pathogens, the MIC values were 20-fold dilutions for the water extracts from fresh tissues of *C. tashiroi* and *Pueraria montana* (PM) against *C. higginsianum*, and 40-fold dilutions for the water extracts from fresh tissues of *C. tashiroi* against *P. infestan*. The MIC values of extracts from dry tissues of *S. nigrum* were 400-fold dilutions against *A. brassicicola*.

Effect of medicinal plant extracts on control of plant diseases

Plant extracts showing strong inhibitory effects on spore germination of fungal pathogens *A. brassicicola*, *C. higginsianum* and *P. infestans* were further assessed for effectiveness in the control of diseases caused by these pathogens, using a detach-leaf technique. Results showed that treating leaves of Chinese cabbage with extracts from fresh tissues (10% (w/v) aqueous extracts) or dry tissues (1% (w/v) aqueous extracts) of *S. nigrum* significantly (P <0.05) reduced the severity of black leaf spot caused by *A. brassicicola* (Fig. 1). The disease severity on inoculated leaves was below 27% for all the treatments with aqueous extraction or ethanol extraction of fresh or dry tissues, compared to 97.2% in the treatment of water control and 97.2% in the treatment of ethanol control.

Seven medicinal plant extracts, two from *C. tashiroi*, three from *P. montana* and two from *Uraria crinita* (UC), were tested as 10% (w/v) aqueous extracts from fresh tissues or 1% (w/v) aqueous extracts from dry tissues for control of anthracnose of Chinese cabbage caused by *C. higginsianum* (Fig. 2). Results showed that treatment of leaves of Chinese cabbage with water extracts from fresh or dry tissues of *C. tashiroi*, cool or hot water extracts from fresh tissues of *P. montana*, or hot water extract from fresh tissues of *U. crinita*, caused a significant (P <0.05) reduction in severity of anthracnose. Among these treatments, water extract from

Table 6. Minimum inhibitory concentration (MIC) against plant pathogens in extracts from medicinal plants

Pathogen[1]	Plant[2]	MIC (-fold)[3]					
		Fresh tissues[4]			Dry tissues		
		Water	Hot water	Ethanol	Water	Hot water	Ethanol
ABA-31	SN	10	20	160	400	100	400
PA-01	CT	20	nd	nd	200	nd	nd
PA-01	PM	20	10	nd	100	nd	nd
PA-01	UC	nd[5]	10	nd	200	nd	nd
PI-109	CT	40	10	10	200	nd	nd

[1] Abbreviations of the plant pathogens were listed in Table 2.
[2] Abbreviations of the medicinal plants were listed in Table 1. Medicinal functions of these plant species were also listed in Table 1.
[3] Minimum inhibitory concentration (MIC) was assessed by spore germination of ABA-31, PA-01, and PI-109.
[4] Solvents for extract preparation: distilled water (25℃), hot water (80℃), and 80% (v/v) ethanol (final concentration was adjusted to 15% (v/v)).
[5] nd: No data. There was no inhibitory effect at the maximum concentration (10-fold of fresh or 100-fold of dry extracts) in this experiment.

Table 7. Effects of aqueous or ethanol extracts from fresh or dry tissues of *Clematis tashiroi* on control of late blight of tomato[1]

Solvent	Disease suppression[2]							
	Extract from fresh material (%)[3]				Extract from dry material (%)			
	0	2.5	5	10	0	0.25	0.5	1.0
Water	—	+	+	+	—	—	+	+
Hot water	—	—	—	+	—	—	—	—
Ethanol	—	—	—	+	—	—	—	—

[1] Tomato leaves treated with plant extracts and inoculated with *Phytophthora infestans* (isolate PI-109) and untreated leaves inoculated with the pathogen (controls) were incubated at 20℃ for 7 days before assessing for diseases incidence.
[2] Disease suppression: +, complete suppression with no lesions developed on inoculated leaves; —: non-suppression, lesions developed on inoculated leaves.
[3] Concentrations of extracts: 0, 2.5, 5, and 10% for fresh tissues and 0, 0.25, 0.5, and 1% for dry tissues.

fresh tissues of *P. montana* was the most effective treatment which reduced the disease severity to 22.2%, compared to 94.4% for both water and ethanol controls (Fig. 2). No significant reduction in disease severity was observed for the treatments of water extracts from dry tissues of *P. montana* (63.9%) and dry tissues of *U. crinita* (88.9%).

Extracts of *C. tashiroi*, were also evaluated for control of tomato late blight caused by *P. infestans* (Table 7). Results showed that water and ethanol extracts from fresh tissues were effective in reducing incidence of late blight at a concentration of 10% (w/v), whereas water extracts from dry tissues were effective at a concentration of 1% (w/v). Meanwhile, *C. tashiroi* at the lower concentrations of 2.5% (w/v) from fresh materials or 0.5% (w/v) from dry materials remained effective in complete suppression of lesion development of *P. infestans* on tomato leaves (Table 7).

DISCUSSION

Locher *et al*. (1995) [21] reported that some ethnobotanical medicinal plants grown in Hawaii contain antimicrobial substances. In our study, plants possessed antimicrobial properties were also found in some species of ethnobotanical medicinal plants used by the indigenous Tsou people in Taiwan. Of the total of 84 plant extracts from 14 species of plants examined, 39 inhibited spore germination (Table 3) a 4 inhibited mycelial growth (Table 4) of fungi, whereas inhibited the growth of bacteria (Table 5). However, antimicrobial effect varied with species of plants, species microorganisms, and extraction methods. For example, pl extracts from *S. nigrum* were highly effective in inhibitior spore germination of *A. brassicicola* and *C. higginsianum* were ineffective against spore germination of *C. scopariu F. moniliforme*, and *P. infestans*. Previous reports indicate t species of plants, organs of plants and the solubility of inhibitory compounds in the solvents may be major fact affecting the extraction of antimicrobial compounds fr plant tissues [2, 10, 12, 30]. Therefore, it is important to iden the chemical nature of the toxic components in the pl extracts that showed strong antimicrobial activities.

Extracts of *S. nigrum* appears to be toxic to numer species of microorganisms. Besides the inhibitory effect or *brassicicola*, *C. higginsianum* and *R. solani* observed in t study (Tables 3, 4), *S. nigrum* was also reported to cont substances that are suppressive to other plant pathogens su as *Alternaria solani* (Ell. & Mart.) Jones & Gro *Helminthosporium sativum* Panm., King & Bakke, and *solani* [28]. Alvarez-Castellanos *et al*. (2001) [1] reported t essential oil from garland chrysanthemum (*Chrysanthem coronarium* Willd.) contained natural substances that

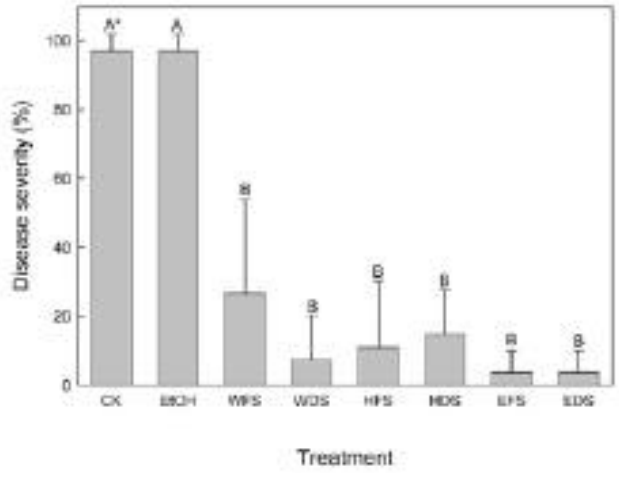

Fig. 1. Effect of water or ethanol extracts from fresh or dry tissues of *Solanum nigrum* applied at 10% or 1% (w/v) on disease severity of black leaf spot of Chinese cabbage caused by *Alternaria brassicicola* (isolate ABA-31). The treated Chinese cabbage leaves and untreated controls were incubated for 3 days at 24℃ and scored for disease severity. Treatments were: CK, sterile distilled water as a control; EtOH, 15% (w/w) ethanol as another control; WFS, water extract of fresh material; WDS, water extract of dried material; HFS, hot water extract of fresh material; HDS, hot water extract of dried material; EFS, ethanol extract of fresh material; EDS: ethanol extract of dried material. Vertical bars = standard deviations;* Means among the eight treatments followed by the same letter are not significantly different ($P > 0.05$) according to Tukey's studentized range test.

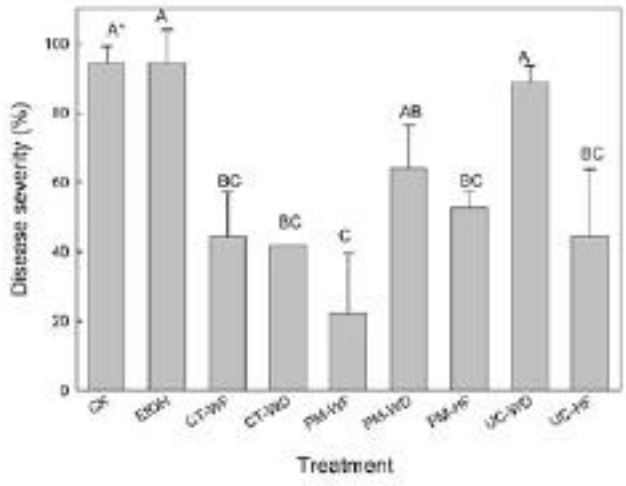

Fig. 2. Effect of extracts from fresh or dry tissues of *Clem tashiroi* (CT), *Pueraria montana* (PM) and *Uraria crin* (UC) applied at 1% (w/v) on disease severity of anthracn of Chinese cabbage caused by *Colletotrichum higginsian* (isolate PA-01). The treated leaves and untreated contr were incubated for 3 days at 24℃. and scored for dise severity. Treatments were: CK, sterile distilled water a control; EtOH, 15% (w/w) ethanol as another control; V water extract of fresh material; WD, water extract of dr material; HF, hot water extract of fresh material; HD, water extract of dried material. Vertical bars = stand deviations;* Means among the nine treatments followed the same letter are not significantly different ($P > 0.$ according to Tukey's studentized range test.

inhibitory to 12 species of plant pathogens, including *A. brassicicola*, *F. moniliforme*, and *R. solani*. Whether the inhibitory effect of the pathogens *A. brassicicola* and *R. solani* by tissue extracts from *S. nigrum* of this study and essential oil from garland chrysanthemum [1] was due to same chemical compounds remains unknown and thus, warrants further investigations.

The inoculation tests using detached leaves reveal that water or ethanol extracts from fresh or dry tissues of *S. nigrum* are effective in reducing severity of black leaf spot of Chinese cabbage caused by *A. brassicicola* (Fig. 1) and the water extracts from fresh tissues of *Clematis tashiroi*, *Pueraria montana* and *Uraria crinita* are effective in reducing severity of anthracnose of Chinese cabbage caused by *C. higginsianum* (Fig. 2). Water extracts from fresh or dry tissues of *C. tashiroi* are also effective in reducing incidence of late blight of tomato caused by *P. infestans* (Table 7). Most of the abstracts remain effective when applied at low concentrations. Therefore, the ethnobotanical medicinal plants *C. tashiroi*, *P. montana* and *U. crinita* may be the potential source of materials for use in the development of natural products for control of fungal diseases of Chinese cabbage and tomato. Although the use of ethnobotanical medicinal plants for treatment of human illness has been well documented [3, 21, 26, 29], reports on the use of these plants for control of plant diseases are rare. Thus, further research efforts in developing safe natural disease control products for practical application would be rewarding.

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摘 要

武藤真知子[1]、高橋久光[1]、石原邦[1]、湯淺浩史[2]、黃振文[3,4]. 2005. 台灣原住民常用之藥用植物抽出物的抗菌活性. 植病會刊 14: 13-24. ([1.]日本東京農業大學國際農業發展學系；[2.]日本東京農業大學環境綠地學系；[3.]台中市國立中興大學植物病理學系；[4.]聯絡作者，電子郵件：jwhuang@dragon.nchu.edu.tw ；傳真：+886-4-22851676)

利用*Alternaria brassicicola*、*Colletotrichum higginsianum*、*Cylindrocladium scoparium*、*Fusarium moniliforme*、*Phytophthora infestans*、*Pythium aphanidermatum*及*Rhizoctonia solani* 等七種植物病原真菌，與*Erwinia carotovora* Subsp. *carotovora*、*Pseudomonas syringae*及*Xanthomas campestri* pv. *vesicatoria* 等三種植物病原細菌，評估十四種台灣原住民鄒族常用藥用植物 (新鮮與乾燥) 之水溶液與酒精抽出物的抗菌活性，藉以作為研發植物病害防治新策略的基礎。本研究發現植物抽出物抑制孢子發芽及菌絲生長和細菌細胞增殖的效果，會隨植物種類的不同而有明顯的差異。在所有參試的樣品中，龍葵 (*Solanum nigrum*) 的水溶液與酒精抽出物均能完全抑制十字花科蔬菜黑斑病菌 (*A. brassicicola*) 的孢子發芽，顯示它是一種最具有抗真菌活性的植物。新鮮龍葵酒精抽出物抑制 *A. brassicicola* 的最小抑制濃度 (MIC) 是 160 倍稀釋液；而乾燥龍葵水溶液與酒精抽出物的 MIC 則均是 400 倍稀釋液。針對其他病原菌的研究分析，發現新鮮琉球鐵線蓮 (*Clematis tashiroi*) 與新鮮山葛 (*Pueraria montana*) 水溶液抽出物抑制白菜炭疽病菌 (*C. higginsianum*) 的 MIC 是 20 倍稀釋液；至於新鮮琉球鐵線蓮水溶液抽出物抑制番茄晚疫病 (*P. infestans*) 的 MIC 則是 40 倍稀釋液。進一步，利用切離葉感染法分析植物抽出物的防病功效，發現新鮮龍葵酒精抽出物與新鮮山葛水溶液抽出物分別可以顯著 ($P < 0.001$) 降低十字花科黑斑病與白菜炭疽病的發病百分率。其中新鮮龍葵酒精抽出物防治十字花科黑斑病的效果可從對照 97.2% 降到 3.7%；而新鮮山葛水溶液抽出物防治白菜炭疽病的效果，則可由對照 94.4%降到 22.2%。此外，新鮮與乾燥過的琉球鐵線蓮水溶液抽出物分別在 2.5 與 0.5% (w/v) 濃度可以完全抑制番茄晚疫病的發生。本研究證明植物抽出物具有應用於防治作物病害的潛力；未來也許可以研發成為田間防治作物病害的天然物植保製劑產品。

關鍵詞：生物防治、原住民之藥用植物、植物源農藥、植物抽出物