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Antimicrobial and weed inhibitory activities of *Senna spectabilis* extracts against plant pathogens

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Abstract *Senna spectabilis* or *Cassia spectabilis* belongs to Leguminosae-Caesalpinioideae is widely grown as an ornamental plant in Thailand. Antifungal and antibacterial of plant disease and herbicidal activities of *S. spectabilis* crude extracts were studied. The flowers and leaves extracts of *S. spectabilis* displayed low to moderate inhibit *Colletotrichum gloeosporioides* and *Fusarium oxysporum* (1.85-44.44%) at 1,000 ppm. The flowers extracts from dichloromethane was showed the highest against *Rhizoctonia sola*ni (47.04%). However, these extracts could not inhibit the mycelial growth of *Phythophora parasitica*. Moreover, methanol stem extract inhibited *Erwinia chrysanthemi* and *Xanthomonas axonopodis* at 16.00±1.20 and 25.00±5.00 mm at 10,000 ppm, respectively. Furthermore, the methanol extract from leaves and dichloromethane extract from flower of *S. spectabilis* at 10,000 ppm were completely inhibited the germination, the growth of shoots and roots of Swollen finger grass (*Chloris barbata*) and followed by methanol extracts of flower and stem with 95.50% germination inhibitory. While the seed germination of Chinese Cabbage-PAI TSAI (*Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee) was inhibited by dichloromethane flower extract at 71.38%. Moreover, the growth of Chinese Cabbage-PAI TSAI was completely inhibited by methanol leaves and flower extracts from this plant. This research was demonstrated the potential of *S. spectabilis* to inhibit plant pathogens, weed germination and growth. These extracts should be applying to agriculture to control weed and microbial plant pathogens for reduce chemical use and non-toxic to the environment.

Keywords: *Senna spectabilis*, plant disease, germination, herbicidal activity

Introduction

Agriculture has been with Thai society since the ancestors until now. Thailand is agricultural country rich in natural resources. In Phetchaburi and Prachubkhirikhan province, most of the population consists of farmers. There are monoculture and integrated farming such as rice, pineapple, banana, mango,

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lime, coconut, jackfruit, plam oil and some vegetable crops. Modern agriculture technology, fertilizers and pesticides are introducing for increase the quantity and quality of the produce to serve as consumers, processing industry and export. In order to generate income and promote the country economic prosperity are expected. However, the production from agriculture was attracked by pest including fungi, bacterial, insect and weed. Weed caused barriers to work such as interrupting drainage, fertilizer management, tillage harvesting and hiding of insects (Smitt *et al*., 2000). In addition, many plant diseases are caused by fungi and bacteria such as wilting and some grain discoloration disease, antracnose, pineapple heartrot, root rot are caused by *Fusarium* spp., *Colletotrichum gloeosporioides, Phytophthora parasitica* and *Rhizoctonia solani*, respectively. The bacteria, *Xanthomonas* sp. and *Erwinia* sp. are caused canker and vegetable decray, respectively (Agrios, 2005). These pests are a bargain of food and area for crops, plant growth, quality and yield of crops decrease.

For pests management, Thailand imported a large number of pesticide chemicals to control and solve thease problem. Even to the cost, toxicity of these pesticide chemical affected to farmer and consumer health. The residues of pesticide were affected the environment around crops plantation area (Colbach and Meziere, 2013). Moreover, the associations of pesticide usage with development of fungicide-resistant strains and nowadays, the concern about human health and the environmental pollution was stimulated the search for new strategies as alternative means for controlling plant disease (Sukatta et al, 2008). Biological pesticides are formulations made from natural substances and used for control pests. This biological pesticide derived from plant, animal and microorganism that can control pests in agriculture system (Anuagasi *et al*., 2017).

Thailand is located in the tropical region with vast of biodiversity and abundance of medicinal plants and used for agrochemicals. And plants have long been recognized as a potential source of bioactive compounds, which may be used against plant pathogens microbial and weed (Parveen *et al*., 2013). *Senna spectabilis* or *Cassia spectabilis* (family Fabaceae, subfamily Leguminosae-Caesalpinioideae) is widely grown as ormamental tree in tropical and subtropical areas. This plant has known as American cassia or in Thai call Khe Lek American. It is widely grown as an ornamental plant in Thailand. This plant has been used in traditional medicine for the treatment of laxative, purgative, flu and cold (Sangetha *et al*., 2008a). The leaf of *S. spectabilis* extract in methanol showed against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas auroginosa* and *Candida albicans* with MIC 0.625-2.5 mg/ml and MBC/MFC 1.25-5.00 mg/ml

(Krisshnan *et al*., 2010). The extracts from flower, leaf and stem of *S. spectabilis* exhibited antioxidant activity, antimalarial, antifungal activity against *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* (Pivatto *et al*., 2016; Sangetha *et al*., 2008a, b, c).

Until now, the available data of *S. spectabilis* are deficient as antimicrobial plant pathogens. The aims of this study was carried out to test antimicrobial from several parts of *S. spectabilis* and to test herbicidal activity against weed and crop seed germination and growth.

Materials and methods

Preparation of plant extracts

The fresh leaf, flower and stem of *S. spectabilis* were collected from Silpakorn University, Cha-Am, Phetchaburi, Thailand in 2017. The plant material was examined and washed to remove dirt before dried in oven 60 C for 3 days and ground into powder. The poder material were extracted by Soxhlet apparatus start with dichloromethane (DCM), after remove DCM and concentrated with rotary evaporator to receive DCM extract. The redidual was further extracted with methanol (MeOH) and concentrated with rotary evaporator to receive MeOH extract.

Antifungal assay

The DMC and MeOH extracts from several parts of American cassia were investigated for antifungal activity against four fungi: *Colletothrichum gloeosporioides* DOAC 2047, *Fusarium oxysporum* DOAC 1258, *Phytophthora parasitica* DOAC 2052 and *Rhisoctonia solani* DOAC 1406. These fungi were supplied by Division of Plant Disease and Microbiology, Department of Agriculture, Ministry of Agriculture and Cooperative, Bangkok, Thailand. Food poison technique was used for antifungal test start with the crude extracts from plants were dissolved in acetone after that, 100 µL of each solution extract was aseptically added and mixed with 9.9 mL of PDA to obtain the final concentration of 1,000 ppm. After well-mixed with PDA, it was poured into Petri dish until solidification, disc of tested fungi (6 mm) placed in the center of Petri dish. The agar plates amend with acetone as a negative control. After the tested plates were incubated for 5-7 days at 27˚C. The radial mycelial measurements of growth were taken when the fungi of control plate reached the edge of plate. The colony diameters were measured and calculated

as percentage mycelial growth inhibition according to the formula (Tegegne *el al*., 2008). All treatments were three replicated.

$$\text{Percentage inhibition of mycelial growth (\%)} = [(dc-dt)/\ dc \times 100]$$

dc: average diameter of the fungal colony of control plate
dt: average diameter of the fungal colony of treatment plate

Antibacterial assay

The antibacterial activity of crude extracts and isolated compounds were determined using agar well diffusion method as described by Irshad and co-workers (2012) with some modification. Twenty millilitres of nutrient broth agar (NA) were melted and poured into sterilized Petri dish plate (9 cm diameter). After the agar was allowed to set and harden, the bacterial inoculum was swabbed on NA. The numbers of holes were cut using a sterile cork borer (6 mm) and agar plugs were removed. The 30 µL of extracts or isolated compounds (10,000 ppm) were added into the wells. The tested plates were incubated at 37°C for 24 h. The diameter of zones inhibition (mm) was measured.

Herbicidal assay

The Herbicidal inhibitory assay of crude extracts from several parts of *S. spectabilis* on crop and weed were investigated. The seed of Swollen finger grass (*Chloris barbata*) and Chinese Cabbage-PAI TSAI (*Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee). Each crude extract was dissolved in acetone at the concentration of 10,000 ppm. Two milliliters or each solution were added to sterile Petri dish with filter paper (No .1 Whatman™, 90 mm diameter). After the solvent was completely dried, 5 mL of sterilized water were added into the Petri plate and added and 30 seeds of tested weed and crop were placed on the filter paper for each treatment and control plate as acetone. The plates were incubated for 3-5 days at 27 ℃. The germination, length of hypocotyl, root and fresh weight were recorded. For each treatment 3 replications were done along with acetone as control.

Statistical analysis

The experiment was designed as a Completely Randomized Design with triplicate and expressed as mean ±standard deviation (SD). All data was analyzed by R program (R-language and environment for statistical computing and graphics, version 3.5.1). The difference of means was performed by one-

way ANOVA, Duncan's multiple range test (DMRT) (P<0.05).

Results

Plant extracts

The crude extrats of *S. spectabilis* from leaf flower and stem that extracted with DCM and methanol, methanol leaf extracts displayed the highest percent yild as 16.80% with dark green semi-solid and the yield followed by DCM leaf extract (16.08%) with drak green liquid and the methanol flower extract (12.60%) with brow semi-solid. The other extracts were showed low percent yield between 0.84-6.11%.

Antifungal activity

The antifungal activity of the crude extracts from leaf, flower and stem parts of *S. spectabilis* were tested by food poisonous method at 1,000 ppm. The results showed in Table 1. Among the extracts, the methanol extract from stem part of *S. spectabilis* revealed the highest activity against all of tested fungi except *R. solani*. The percentation of methanol extract from stem part was significant displayed percentage of inhibition as 44.76%, 44.44% and 15.93% against *P. parasitica*, *F. oxysporum* and *C. gloeosporioides*, respectively at 1,000 ppm. However, the flower crude extract in DCM part exhibited the highest against *R. solani* with 47.04% and followed by DCM stem crude extract with 37.78% at 1,000 ppm. However, all crude extracts were stimulated the growth of *P. parasitica* except stem methanol extract.

Table 1. Antifungal activity of *S. spectabilis* crude extracts against plant pathogenic fungi at 1,000 ppm.

Plant parts	Fractions	Percent inhibition (% ±SD)			
		C .gloeosporioides	*F .oxysporum*	*P .parasitica*	*R .solani*
Leaf	DCM	0.00 ±0.00$^{c1/}$	4.81 ±1.115^{c}	-28.57 ±0.00^{d}	0.00 ±0.00^{e}
	MeOH	1.85 ±1.15^{bc}	7.04 ±0.58^{c}	-24.29 ±2.65^{d}	27.41 ±0.58^{c}
Flower	DCM	17.78 ±1.73^{a}	17.78 ±1.73^{b}	-27.14 ±1.99^{d}	47.04 ±2.52^{a}
	MeOH	2.59 ±0.58^{b}	19.26 ±2.31^{b}	-1.90 ±2.31^{b}	22.22 ±4.58^{d}
Stem	DCM	1.48 ±1.15^{bc}	5.19 ±0.58^{c}	-17.62 ±2.08^{c}	37.78 ±1.00^{b}
	MeOH	15.93 ±0.58^{a}	44.44 ±0.00^{a}	44.76 ±1.15^{a}	29.63 ±0.00^{c}

[1]/Indicate significant effects at P<0.05 (DMRT) within a column

Antibacterial activity

The efficiency of antibacterial activity of *S. spectabilis* crude extracts tested by agar well diffusion method against *E. chrysanthemi* and *X. axonopodis* at 10,000 ppm. According to Table 2 the methanol stem extract showed significant the highest activity against both of plant pathogenic bacteria *E. chrysanthemi* and *X. axonopodis* with inhibition zone 16.0 mm and 25.00 mm, respectively. The results antibacterial activity was followed by methanol extracts from leaf, flower parts and DCM leaf extracts inhibited *E. chrysanthemi* with the inhibition zone 13.00, 10.00 and 12.00 mm, respectively. The other crude extracts were displayed not significant with inhibition zone of *X. axonopodis* as 9.7-14.0 mm.

Table 2. Antibacterial activity of S. spectabilis crude extracts against *E. chrysanthemi* and *X. axonopodis* at 10,000 ppm

Plant part	Fractions	Inhibition zone (mm)	
		E. chrysanthemi	***X .axonopodis***
Leaf	DCM	$12.00 \pm 1.70^{bc1/}$	9.70 ± 0.60^{b}
	MeOH	13.00 ± 2.10^{ab}	110.0 ± 0.60^{b}
Flower	DCM	9.70 ± 0.60^{c}	11.00 ± 1.20^{b}
	MeOH	10.00 ± 2.50^{bc}	14.00 ± 3.50^{b}
Stem	DCM	9.30 ± 1.20^{c}	12.00 ± 2.60^{b}
	MeOH	16.00 ± 1.20^{a}	25.00 ± 50.0^{a}

[1]/Indicate significant effects at $P<0.05$ (DMRT) within a column

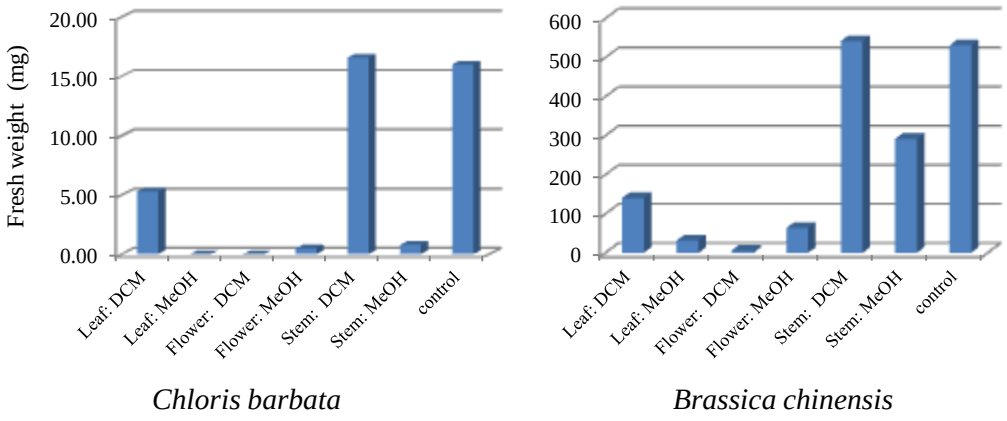
Herbicidal assay

Herbicidal activity of crude extracts from several part of *S. spectabilis* displayed in Table 4. The DCM flower crude extracts and MeOH leaf extract showed excellent inhibition germination, root and shoot of *C. barbata* and radical growth of *B. chinensis*. The methanol extracts from flower and stem were significantly inhibited the germination, root and shoot growth in both of tested weed and crop with >95% inhibition. However, the DCM stem extract not only showed low effective against germination and growth of *C. barbata* but this extract also stimulated the hypocotyl and radical growth of *B. chinensis*. The effective of crude extracts from this plant on fresh weight of *C. barbata* and *B. chinensis* displayed in Figure 1. The flower extract, methanol leaf and stem extracts showed excellent against fresh weight of *C. barbata* and *B. chinensis*.

Table 4. Percentage herbicidal activity of *S. spectabilis* crude extracts inhibit germination and growth of weed and crop seed at 10,000 ppm

Crude extracts	Inhibition of *Chloris barbata* (%)			Inhibition of *Brassica chinensis* (%)		
	Germination	Shoot	Root	Germination	Hypocotyl	Radical
Leaf: DCM	72.71±0.00$^{b1/}$	85.31±7.45^{b}	88.18 ±8.75^{a}	12.66±2.89^{b}	68.09±4.00^{b}	84.48±2.63^{b}
Leaf: MeOH	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	25.28±7.77^{b}	97.33±1.31^{a}	100.00±0.00^{a}
Flower: DCM	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	71.38±3.06^{a}	99.67±0.58^{a}	100.00±0.00^{a}
Flower: MeOH	95.50±0.58^{a}	98.82±2.68^{a}	98.72±1.79^{a}	8.03±0..58^{b}	91.40±1.31^{a}	100.00±0.00^{a}
Stem: DCM	4.50±1.00^{c}	38.82±3.19^{c}	25.56±2.55^{b}	6.90±1.00^{b}	-42.94±5.18^{c}	-46.94±7.82^{c}
Stem: MeOH	95.50±0.58^{a}	96.93±4.59^{a}	96.49±3.53^{a}	1.14±1.53^{b}	34.75±2.88^{b}	99.94±1.74^{a}

[1]/Different lower case letters for each column indicate that the values were compared significantly different at $P<0.05$ (DMRT)



Chloris barbata *Brassica chinensis*

Figure 1. Herbicidal effect of crude extracts from *S. spectabilis* on fresh weight of *Chloris barbata* and *Brassica chinensis* at 10,000 ppm.

Discussion

Plants produce a great diversity of secondary metabolites with a very broad spectrum of biological activity. The *Senna* genus comprises about 600

species distributed worldwide and the extracts from these plants was been reported effects such as antimicrobial anticonvulsant antioxidant anti-inflamatory and sedative (Castro *et al*., 2016). *S. spectabilis* or American cassia is a wooden flowering plant native to Central and South America and has been found in Africa and Asia as ornamental plants. This plant was used for traditional medicine as laxative analgesic antimicrobial insomnia anti-ulcerogenic agents (Silva *et al*., 2011). In Thai folk medicine, the leaf of *S. spectabilis* are used fro treat fever, headaches and various skin infecttions (ringworm, eczema and scabiess) (Singh *et al*., 2013). The previous work reported the leaf of *S. spectabilis* extract in methanol showed against bacterial such as *S. aureus*, *E. coli* and *P. auroginosa* better than the extract from benzene and chloroform (Krisshnan *et al*., 2010). This work also demonstrated that the extract of *S. spectabilis* from methanol part showed extract to against plant pathogens bacteria (*E. chrysanthemi* and *X. axonopodis*) higher than DCM crude extracts at 10,000 ppm.

The antifungal activity of *S. spectabilis* was reported to against *C. albicans* and *A. niger* by disk diffudion assay (Krishnan *et al*., 2010). However, this work is firt report that work with antimicrobial activity against plant pathogens from the extracts of *S. spectabilis*. The results of antifungal activity, the methanol extracts from stem and flower parts showed good activity against all of tested plant pathogens fungi except *P. parasitica*. According to Table 1, all part of *S. spectabilis* crude extracts (except stem methanol extract) stimulated the mycelium growth of *P. parasitica* because the chemical constituent of this plant consist of β-sitosterol and stigmasterol which these compounds were used as power source for growth of *P. parasitica* (Mulchaldani and Hassarajani, 1977).

The herbicidal assay to against weed and some crop test found that the methanol leaf extract and DCM flower extract displayed significat inhibit the germination and growth of *C. barbata* and *B. chinensis* at 10,000 ppm. In 2003, Maclean and co-worker reported the application of fresh leaf from *C. spectabilis* to mulch on the soil surface between furrows plating of upland rice and this plant reduced weed biomass (Maclean et al., 2003).

This work was demonstrated the potential of *S. spectabilis* to inhibit plant pathogens and herbicidal activities. These extracts should be applying to agriculture field to control weed and microbial plant pathogens for reduce aagro-chemical use and friendly with the environment. Moreover, these crude extracts from *S.spectabilis* in the active parts should be further separation and characterization of the active compounds.

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