Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 2 (1): 123-126, 2007
ISSN 1816-4951

Antimicrobial Activity of *Senna spectabilis* and *S. tora*

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Abstract: Crude ethanol and aqueous extracts derived from different parts (i.e., leaf, flower, stem and pod) of *Senna spectabilis* and *S. tora* were tested *in vitro* against *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Saccharomyces cerevisiae*. It was shown that only crude water extracts of *S. spectabilis* could inhibit the growth of *B. cereus* (20-25 mm, diameter of the clear zone). In contrast, both crude ethanol and aqueous extracts of *S. tora* were active against *E. coli* growth (6-10 mm) and only crude water extracts of *S. tora* were able to inhibit the *S. cerevisiae* growth. However, none of these crude extracts could inhibit the growth of *C. albicans*.

Key words: *Senna spectabilis*, *S. tora*, antimicrobial activity

Introduction

Since ancient time, plant and animal products have been used for treatment of diseases and disorders. Plants in particular have been used to treat infectious diseases due to its antimicrobial properties. This is due to the presence of various kinds of phytochemicals including phenolic compounds, alkaloids, terpenoids and essential oils (Lewis and Elvin-Lewis, 1995; Cowan, 1999). Therefore, plant natural products are anticipated to be an alternative source of novel antimicrobial agent finding.

The Genus *Senna* (previously described as *Cassia*) is a pantropical shrub of the family Leguminosae comprising of more than 300 species (Randell and Barlow, 1998). It is widely distributed in the tropical countries such as the USA, India, Thailand, Malaysia, Indonesia and the Australasia region. Traditionally, the sap or the extract of the plant (i.e., root, stem and sicklepod) has been used against ringworm, ulcers and other skin diseases (Seaforth, 1962; Kirtikar and Basu, 1975). Several reports have shown that some *Senna* species exhibit antimicrobial activity. *S. alata* in particular is of great interest due to its broad spectrum on pathogenic microbes (Ibrahim and Osman, 1995; Khan *et al*., 2001; Somchit *et al*., 2003; Phongpaichit *et al*., 2004). Antifungal activity of the extracts derived from *S. fistula* and *S. tora* has also been described (Phongpaichit *et al*., 2004). In addition, an antimicrobial alkaloid derived from *S. racemosa* known as 'cassine' is also found to inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis* (Sansores-Peraza *et al*., 2000).

S. spectabilis (Thai name: Kee Lek American) and *S. tora* (Thai name: Chum Hed Thai) are widely present throughout Thailand as ornamental plants. In traditional medicine, both plant species are used to cure ringworm and skin diseases (Farnsworth and Bunyapraphatsara, 1992). There is not much work however regarding the antimicrobial property of these two plants. Therefore, the aim of the present study was to evaluate the antimicrobial potentiality of the leaf, flower, stem and pod extracts of *S. spectabilis* and *S. tora* against the growth of some pathogenic microbes.

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Materials and Methods

Plant Materials and Extraction

S. spectabilis and *S. tora* were collected from Tasud, Chiang Rai, Thailand during October-November 2004 and the leaves, stem, flowers and pods were separated individually. The plant samples were authenticated by Mrs. Tovaranonte, the botanist of the School of Science, Mae Fah Luang University, Thailand. Parts of *S. spectabilis* and *S. tora* were oven dried at 45°C for 24-48 h and macerated into powder form. Extraction using Soxhlet apparatus with either 95% (v/v) ethanol or distilled water for 24 h was carried out. The resultant extraction was freeze dried for 24-48 h and kept at 4°C until use. In addition, to compare the results of extracting procedure, the grounded samples were also extracted with either 95% ethanol for 48 h or with boiling water for 1 h at room temperature. After filtrating, fractions were evaporated to dryness, freeze-dried and stored in the refrigerator until required.

Microorganisms and Media

The microorganisms used in this study were bacteria (*Escherichia coli* TISTR780 and *Bacillus cereus* TISTR687) and yeasts (*Saccharomyces cerevisiae* TISTR5049 and *Candida albicans* TISTR5239). All microorganisms were obtained from the Microbiological Resources Centre (MIRCEN), Thailand Institute of Scientific and Technological Research, Thailand. For routine culture and maintenance, tryptic soy agar (Difco) and Yeast and Mould agar (Difco) were used for bacteria and yeasts, respectively.

Antimicrobial Activity Test

In this study, agar disc diffusion method was performed to determine antimicrobial activity of the crude extracts (Collins *et al*., 2004). Initially, microbial strains were grown in appropriate media to exponential phase (between 18-24 h). The microbial cell suspensions used as inoculum were then prepared in the range of 10^6-10^8 cells per plate. Sterile 6.0 mm diameter blank disc were impregnated into the crude extracts at the concentration of 75 mg mL^{-1}, placed on the agar and incubated either at 37°C for 24 h for bacteria or 30°C for 24-48 h for yeasts. Impregnated disc with distilled water and 10% ethanol were used as control. Antimicrobial activities were indicated by clear zones of growth inhibition. Minimum inhibitory concentrations (MIC) was also determined using different dilutions of the extracts as follows: 0, 10, 25, 30, 40, 50 and 70 mg mL^{-1}. The lowest concentration which did not show any growth of the tested microorganism was interpreted as the MIC.

Results and Discussion

Traditional use of plants in the Genus *Senna* is often related to treatment of superficial fungal infection. The present study was further explored if the *Senna* plants could be used against other microbial groups. For this, crude extracts of *S. spectabilis* and *S. tora* were prepared from different parts (i.e., flower and leave) using two solvents: water and ethanol. In addition, heat treatment during the extracting procedure was also considered whether this could affect the antimicrobial activity of the crude extracts prepared. For initial screening, 75 mg mL^{-1} of crude extracts were used and results of the antimicrobial activity tests of *S. spectabilis* and *S. tora* are shown in Table 1 and 2, respectively.

It was found that, for *S. spectabilis*, only crude water extracts had an inhibitory effect on *B. cereus* growth. The anti-*Bacillus* activity was found in both flower and leave samples. Although high temperature (by Soxhlet method) seems not to affect the activity of the active compound(s) in the flower parts, this terminates the inhibitory effect of the compound(s) in the leaves. For *S. tora*, crude extracts derived from pods, leaves and stems were able to inhibit the growth of *E. coli*

Table 1: Antimicrobial activity of flower and leaf extracts of *S. spectabilis*

Organisms tested	Parts used[a]	Zone of inhibition (mm) A1[b]	A2[b]	E1[b]	E2[b]
Escherichia coli	F	-	-	-	-
	L	-	-	-	-
Bacillus cereus	F	23	25	-	-
	L	-	20	-	-
Saccharomyces cerevisiae	F	-	-	-	-
	L	-	-	-	-
Candida albicans	F	-	-	-	-
	L	-	-	-	-

[a]F, Flowers; L, Leaves, [b]A1 and A2, aqueous extracts by Soxhlet and non-Soxhlet method; E1 and E2, ethanol extracts by Soxhlet and non-Soxhlet preparation

Table 2: *In vitro* antimicrobial activity of *S. tora* extracts

Organisms tested	Parts used[a]	Zone of inhibition (mm) A1[b]	A2[b]	E1[b]	E2[b]
Escherichia coli	P	9	-	7	9
	L	6	8	9	-
	S	8	-	7	10
Bacillus cereus	P	-	-	-	-
	L	-	-	-	-
	S	-	-	-	-
Saccharomyces cerevisiae	P	8	-	-	-
	L	7	-	-	-
	S	7	-	-	-
Candida albicans	P	-	-	-	-
	L	-	-	-	-
	S	-	-	-	-

[a]P, Pods; L, Leaves; S, Stems, [b]A1 and A2, aqueous extracts by Soxhlet and non-Soxhlet method; E1 and E2, ethanol extracts by Soxhlet and non-Soxhlet preparation

Table 3: Minimum inhibitory concentrations (MICs) of the aqueous extracts of *S. spectabilis*. The antimicrobial test was performed against *B. cereus*

Crude extracts[a]	Concentration (mg mL^{-1}) 25	30	40	50	70
FA1	-	7.67±0.58	18.33±0.58	20	20
FA2	-	8	10.67±1.15	20.67±1.15	23.33±1.53
LA2	-	7.67±0.58	10.67±1.15	21.33±1.53	22.67±2.52

[a]FA1 and FA2, crude extracts from flowers by Soxhlet and non-Soxhlet methods; LA2, crude extracts from leaves by non-Soxhlet preparation, Data shown are mean of diameter of inhibition zones±SD (mm)

and *S. cerevisiae* in which the results were varied depending on the solvents used and the extracting methods. It should also be noted that none of these crude extracts (from both *S. spectabilis* and *S. tora*) could inhibit the growth of opportunistic fungal pathogen *C. albicans*. Due to high inhibition zone of *S. spectabilis* water crude extracts on the food-borne pathogen *B. cereus*, further experiment was performed to determine the MIC. It was found that the MIC of the extracts from both flower and leave samples was 30 mg mL^{-1} (Table 3).

Conclusion

This present study further describes antibacterial property of *S. spectabilis* and *S. tora*. Especially for *S. spectabilis*, its aqueous extracts seem to be effective against the food-borne pathogen *B. cereus* (inhibition zones of 20-25 mm diameter). In contrast, both aqueous and ethanol extracts of *S. tora*, albeit ineffective, show inhibitory effect on *E. coli*. Future studies with purified active compounds may be useful to evaluate the actual antibacterial properties of these plants. In addition, toxicological experiment must also be undertaken to ensure the safe use.

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