Brief Report

DOI: 10.5582/ddt.v5.1.12

Antibacterial activities of *Sesbania grandiflora* extracts

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ABSTRACT: In this study, *Sesbania grandiflora*, a plant in the Leguminosae family, was investigated for its antibacterial activities. The agar well diffusion assay as well as the agar and broth dilution assays were used for determination of antibacterial activities. The crude ethanolic extracts obtained from different parts of this plant exhibited different potent activities. The stem bark has the most potential to yield an extract with the highest antibacterial action. The fractionation of the stem bark with different solvents indicated that the fractionated extracts obtained from ethyl acetate or butanol possessed the most pronounced antibacterial activity. The kinetic study of bactericidal activities revealed that the butanol fractionated extract of the stem bark was effective against Gram negative bacteria. This study suggests that the stem bark of *S. grandiflora* contains promising antibacterial substances for clinical purposes.

Keywords: *Sesbania grandiflora*, antibacterial activity, crude extract, pathogenic bacteria, killing kinetics

1. Introduction

In Thailand and other Asian countries like China, Japan, and India, medicinal plants are widely used by all sections of the population either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. The world health organization recently accepted an inventory of more than 20,000 species of medicinal plants. Thai medicinal plants and their products are used to control diverse diseases and disorder symptoms such as cough, fever, bronchitis, itching, pneumonia, ulcers, and diarrhea. Researchers are hence increasingly turning their attention to medicinal plants looking for new bioactive or lead compounds to develop better drugs against several degenerative diseases like cancer, diabetes, Alzheimer's, arteriosclerosis as well as diseases caused by pathogenic microorganisms (*1*,*2*).

From previous studies reported by different research groups, it was demonstrated that many naturally occurring compounds found in plants have been shown to possess biological activities such as antibacterial (*3*-*5*), antifungal (*3*,*6*,*7*), antiviral (*3*,*8*), anti-allergic (*4*), anti-cholinesterase (*5*), antioxidant (*9*-*11*), anti-inflammatory (*4*,*5*,*10*), antitumor (*12*,*13*), anti-tyrosinase (*14*), anti-plasmodial (*15*), and cytotoxic effects (*10*,*15*).

The plant genus *Sesbania*, family Leguminosae, is comprised of about 60 species which are widely distributed throughout tropical and subtropical regions. Most species are annual herbs or shrubs, but a few are small trees (*16*). Many species of *Sesbania* are used for soil improvement as green manners or agroforestry trees (*17*). *Sesbania grandiflora*, syn. *Aeschynomene grandiflora*, is a small erect tree that grows rapidly, is a sparsely branched tree that provides light shade, and is often grown as an ornamental (*18*). *S. grandiflora* is widely used in Thailand both for food and medicine. Its leaves and flowers are utilized as food whereas its stem has been long used as a traditional medicine for treatment of ulcers in the oral cavity. It was reported that all parts of *S. grandiflora* are utilized for medicine in Southeastern Asia and India including preparations derived from the roots, bark, gum, leaves, flowers and fruits. In the Philippines, the stem bark of this plant has been used for the treatment of thrush and infantile disorders of the stomach. In Cambodia, the pounded bark is applied to scabies. The juice of the leaves is considered anthelmintic and tonic and is used to treat worms, biliousness, fever, gout, itchiness, and leprosy (*19*). It was also reported that the root of this plant could be applied externally as a poultice against inflammation. The semisolid mass containing powdered roots of *S. grandiflora* in an appropriate amount of water demonstrated a decrease of rheumatic swelling when applied externally with moderate rubbing to the lesion (*20*). Although *S. grandiflora* has been used for treatment in many diseases, most of the biological activities have not yet been adequately evaluated.

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The present study deals with the investigation of antibacterial activity of *S. grandiflora*. Several parts of the whole plant such as leaves, branches, stem barks, and stem cores were screened for their potency on growth inhibition of bacteria. The pathogenic organisms were selected for the study on the basis of their clinical and pharmaceutical importance as well as their potential to cause contamination of food and drugs. The high potent activity extracts of the most potential parts of the plant were further investigated deeply on bacterial killing kinetics.

2. Materials and Methods

2.1. *Plant materials*

S. grandiflora samples were collected in fresh condition from local areas of Northern Thailand. The plant was identified and deposited in the Herbarium of the Faculty of Pharmacy, Chiang Mai, Thailand.

2.2. *Preparation of plant extracts*

2.2.1. *The crude extracts*

Fresh material leaves, branches, stem bark, and stem core were collected and washed with distilled water. Samples were cut into small pieces and dried at 50°C for 24 h. The dried samples were pulverized into powder, and then macerated with 95% ethanol for 48 h × 3 at room temperature. The filtrates from the same part were pooled together and subjected to the rotary evaporator at 45°C and under vacuum for solvent removal. The crude extracts obtained were investigated further for antibacterial activity.

2.2.2. *Fractionation of the extracts*

The dried powder of stem bark was used in this study because its crude ethanolic extract showed the highest antibacterial activity. The plant powder was first macerated in hexane for 48 h × 3 at room temperature. The residue after the third filtration was dried at room temperature to ensure hexane was completely removed. The dried residue was further macerated respectively in three solvents as follows; ethyl acetate, butanol and methanol, in the same manner as hexane. The filtrates of the same solvent were pooled together. The solvent was removed using a rotary evaporator at 45°C under vacuum. The fractionated extracts obtained were subjected to antibacterial activity tests.

2.3. *Bacterial strains*

Standard strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* DMST 5784, *Shigella sonnei* DMST 561 and clinical pathogenic bacterial strains of *Bacillus cereus*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Staphylococcus epidermidis* obtained from the Faculty of Medicine, Chiang Mai University, Thailand were used throughout the study. The bacteria were sub-cultured individually on fresh Tryptic Soy Agar (TSA) and incubated overnight at 37°C. Each bacterial strain was then suspended in 0.9% NaCl, and diluted to a McFarland turbidity standard No. 0.5 (*21*). This bacterial suspension was used for testing antibacterial activity.

2.4. *Determination of antibacterial activities by agar diffusion*

The bacterial inhibitory effect of *S. grandiflora* extracts was carried out by standard agar well diffusion method (*21*). The TSA plate was seeded with the NS suspension of different bacteria. In each plate, a 6 mm diameter well was cut out using a sterile cork borer. Using a sterile autopipette, 50 µL of the proper diluted extract was carefully added into the wells. The same volume of 50% ethanol was used as a negative control. The plates were incubated overnight at 37°C. The antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ). The experiment was carried out in triplicate and the means of diameter of the inhibition zone were calculated.

2.5. *Determination of minimum inhibitory concentration (MIC)*

2.5.1. *Agar dilution method*

The MIC of fractionated extract of the stem bark was determined against the test pathogenic bacteria by a standard agar dilution method (*22*). Briefly, to the tubes containing 9 mL melted Mueller-Hinton Agar (MHA) media, serial dilutions of the extract were added to 10 mL and poured into a sterile plate. Sample concentrations in the agar plates were in the range of 0.63-20.00 mg/mL. Equivalent amounts of sterile distilled water and sterile 50% ethanol were used as controls. Ten µL of each bacteria suspension with turbidity equivalent to McFarland No. 0.5 was inoculated into each agar dilution plate. The inoculated plates were incubated overnight at 37°C. The MIC was defined as the lowest concentration of the samples which prevented this change and exhibited complete inhibition of bacterial growth (*23*).

2.5.2. *Broth dilution method*

The MIC was also determined by using a broth dilution method (*24*). Briefly, the extract was first dissolved in sterile 50% ethanol. The sample obtained was added

to Mueller-Hinton Broth (MHB). This extract solution was serially diluted two-fold with MHB to obtain the extract concentration in a range of 0.63-10.00 mg/mL. Each bacterial suspension obtained from a McFarland turbidity standard No. 0.5 was inoculated into the broth dilution tube. The tubes were incubated overnight at 37°C. Negative controls were prepared with un-inoculated bacterial suspension and the positive controls contained inoculated bacterial suspension without the test samples. After incubation overnight at 37°C, the micro-dilution tubes were checked visually to detect growth inhibition of the bacteria. The growth end points were determined by comparing the amount of growth in the tubes containing the test samples with that in the negative control. Acceptable growth must occur in the positive control. The MIC was defined as the lowest concentration of the samples which is able to inhibit any visible bacterial growth (*25*).

2.6. *Determination of minimum bactericidal concentration (MBC)*

To determine MBC, a method of MIC determination was slightly modified. Briefly, the samples were taken from the tube of the MIC assays, where no visible turbidity (growth) was observed, and streaked on freshly prepared TSA plates. The plates were incubated overnight at 37°C so as to determine the MBC (*26*). The MBC was defined as the lowest concentration of the samples with no bacteria growth on the surface of the medium in the plates (*27*,*28*).

2.7. *Kinetics of bacteria killing*

Kinetics of bacteria killing was investigated by a broth dilution method (*29*). Briefly, an overnight culture in TSA was adjusted with normal saline (0.9% NaCl) to a McFarland Standard No. 0.5. The adjusted culture was added to MHB with the test extract at the same concentration of MBC and incubated for an appropriate time of 1.5, 3, 4.5, and 6 h, at 37°C, to achieve a logarithmic growth phase. All cultures contained approximately 1.5×10^6 CFU/mL at the initiation of the time course and were maintained at 37°C. The actual initial concentrations were determined by ten-fold serial dilution with a normal saline solution of the inoculums and plating the serial dilutions on TSA to calculate the logarithm of colony forming units per milliliter extract (log CFU/mL). At 0, 1.5, 3, 4.5, 6, and 24 h, samples were removed and serially diluted with normal saline. The log CFU/mL in the culture at each time point was determined by spreading on freshly prepared TSA plates and incubating overnight at 37°C. As a general rule, plates containing greater than 30, but less than 300 colonies were used to quantify viable CFU/mL. In some instances, the resulting number of colonies was less than 30 and considered below the limit of quantification. In these few cases, the colonies were counted and the resulting CFU/mL was used to provide an estimate of the number of viable cells. It is noted in the appropriate figure legends when these estimates were used. Results of killing kinetic studies are expressed as the difference between the log CFU/mL at the indicated time point and the log CFU/mL of the inoculums at time zero. Bactericidal activity was defined as decreasing the original inoculums after 24 h of incubation (*30*).

3. Results and Discussion

The crude extracts of 4 parts of *S. grandiflora* showed different yields. The leaf extract gave the highest yield of 23.3% (w/w) followed by the stem bark with a yield of 13.3% (w/w). All of them were first screened for their antibacterial activity against *E. coli* and *S. aureus* as representations for Gram negative and Gram positive bacteria, respectively. Gentamicin at a concentration of 75 µg/mL was used as a positive control. It was found as shown in Table 1 that the stem bark extract showed the strongest inhibition against both pathogens with a DIZ of 9.4 and 13.7 mm for *E. coli* and *S. aureus* respectively whereas the leaf extracts gave no inhibition zone.

MIC of the fractionated extracts of the stem bark was further examined against ten strains of pathogenic bacteria. The results in Table 2 indicated that the extract with ethyl acetate possessed the strongest antibacterial activity followed by the butanol extract. The MIC of ethyl acetate extract against all tested pathogens was lower than other solvents. It was found that the yield of ethyl acetate extract was 1.75% which was a little lower than that of the methanol extract but higher than that of the butanol and hexane extracts. Considering the antibacterial activity, the ethyl acetate and butanol extracts were selected for further investigation.

The antibacterial activity in the ethyl acetate and the butanol extracts determined by agar dilution method were confirmed by using the broth dilution

Table 1. Diameter of inhibition zone of growth inhibition of crude extract (100 mg/mL) from each part of *S. grandiflora* and gentamicin (75 µg/mL) against different bacterial strains using the agar well diffusion method[a]

Samples	Diameter of inhibition zone (mm)[b]	
	E. coli	*S. aureus*
Leaves extract	NZ[c]	NZ
Branches extract	7.8 ± 0.3	10.7 ± 1.0
Stem bark extract	9.4 ± 0.1	13.7 ± 0.6
Stem core extract	NZ	8.9 ± 0.2
Gentamicin	16.3 ± 0.9	23.1 ± 2.5

[a] No bacterial growth in the negative control plate.
[b] Data are presented as means ± S.D. (n = 3).
[c] NZ represents no inhibition zone.

method. In this experiment, *B. cereus*, *E. faecalis*, *S. aureus*, and *S. epidermidis*, as representatives for Gram positive bacteria and *S. typhi* and *S. sonnei*, as representatives for Gram negative bacteria were used as test microorganisms. The results shown in Table 3 demonstrate that the MIC values determined by broth dilution method were equal or less than those obtained by the agar dilution method. Moreover the results indicated that the MBC values of extracts against the Gram positive bacteria were equal to the MIC values on corresponding bacteria. Regarding the Gram negative bacteria, it was found that the MBC values were higher than the MIC values on corresponding bacteria.

To study the killing kinetics effect of the highest potential antibacterial activities, the ethyl acetate and the butanol extracts with a concentration at MBC, were subjected to test four pathogens including *E. faecalis*, *S. aureus*, *S. typhi*, and *S. sonnei*. In this experiment *E. faecalis* and *S. aureus* were used as the model of Gram positive bacteria whereas *S. typhi* and *S. sonnei* were used as the model of Gram negative microorganisms. The bactericidal kinetics was examined using time course experiments measuring the number of surviving bacteria, Log CFU/mL. It was seen that both extracts possessed a similar killing rate against the tested Gram positive bacteria. Both extracts showed killing activity against *E. faecalis* within 30 min (Figure 1A). For *S. aureus*, more than 90% of cells were killed within 1.5 h (Figure 1B). It was found that the butanol extract had a high ability to kill *S. typhi* and *S. sonnei*, the tested Gram negative bacteria, faster than the ethyl acetate extract. The results demonstrated that both extracts could kill *S. typhi* and *S. sonnei* within 1.5 h. However, the butanol extract showed complete inhibiton against both strains at 6 h whereas the complete killing time

Table 2. The agar dilution method was used to determine MIC of the extract with different organic solvents from stem bark of *S. grandiflora* against different bacterial strains[a]

Microorganisms	MIC for test samples (mg/mL)			
	Hexane	Ethyl acetate	Butanol	Methanol
Bacillus cereus	> 20	2.5	2.5	2.5
Enterobacter cloacae	> 20	5	20	> 20
Enterococcus faecalis	20	5	5	10
Escherichia coli	> 20	5	20	> 20
Pseudomonas aeruginosa	> 20	10	20	20
Salmonella typhi	> 20	5	2.5	10
Serratia marcescens	> 20	10	20	> 20
Shigella sonnei	> 20	5	5	20
Staphylococcus aureus	10	2.5	2.5	5
Staphylococcus epidermidis	20	2.5	2.5	10

[a] No bacterial growth in the negative control plate.

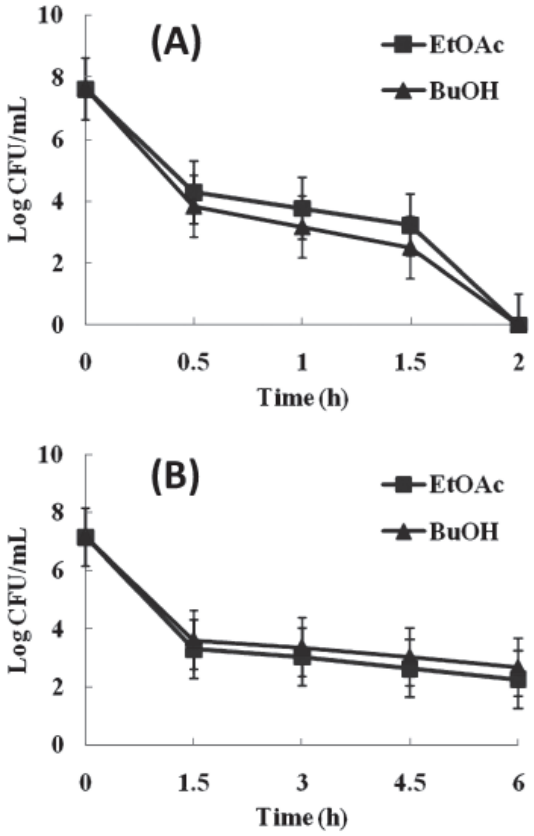

Figure 1. Killing kinetic curves of Gram positive bacteria, *E. faecalis* (A) and *S. aureus* (B), with ethyl acetate (EtOAc) and butanol (BuOH) extracts, from stem bark of *S. grandiflora*.

Table 3. The broth dilution method was used to determine MIC and MBC of the ethyl acetate and the butanol extracts from stem bark of *S. grandiflora* against some different bacterial strains[a]

Microorganisms	MIC for test samples (mg/mL)		MBC for test samples (mg/mL)	
	Ethyl acetate	Butanol	Ethyl acetate	Butanol
Bacillus cereus	1.25	1.25	1.25	1.25
Enterococcus faecalis	5	5	5	5
Salmonella typhi	1.25	1.25	2.5	2.5
Shigella sonnei	2.5	2.5	5	5
Staphylococcus aureus	2.5	2.5	2.5	2.5
Staphylococcus epidermidis	2.5	2.5	2.5	2.5

[a] No bacterial growth in the negative control plate.

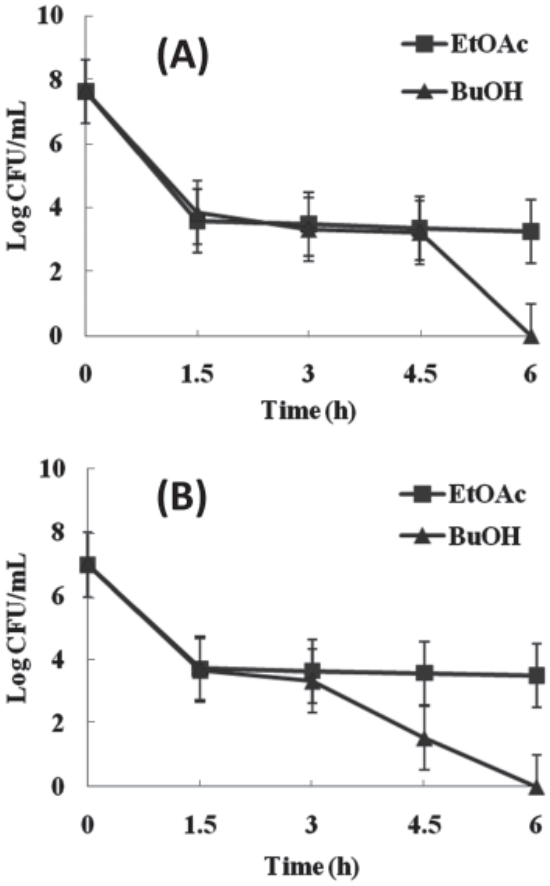

Figure 2. Killing kinetic curves of Gram negative bacteria, *S. typhi* (A) and *S. sonnei* (B), with ethyl acetate (EtOAc) and butanol (BuOH) extracts, from stem bark of *S. grandiflora*.

of ethyl acetate extract for Gram negative bacteria was more than 6 h (Figure 2). From these results, it could be clearly seen that the butanol extract had a more pronounced killing rate on Gram negative bacteria than the ethyl acetate extract. The killing kinetics of both extracts on Gram positive bacteria was not distinguishable.

Acknowledgements

The authors are grateful the RGJ Grant for financial support and the Graduate School, Chiang Mai University for their support. We also thank the Faculty of Medicine, Chiang Mai University, Thailand for kindly providing the tested clinical pathogenic bacteria.

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(Received January 6, 2011; Revised January 17, 2011; Accepted January 19, 2011)