ฤทธิ์ต้านแบคทีเรียของสารสกัดจากเปลือกต้นแคบ้านต่อแบคทีเรียก่อโรคท้องเสีย
Antibacterial Activity of *Sesbania grandiflora* (L.) Desv. Bark Extract against Diarrheal Pathogens

นิพนธ์ต้นฉบับ

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Original Article

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาฤทธิ์ของสารสกัดจากเปลือกต้นแคบ้านในการยับยั้งเชื้อแบคทีเรียก่อโรคอุจจาระร่วง 5 สายพันธุ์ คือ *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* DMST 15676, *Shigella sonnei* ATCC 11060 and *Vibrio parahaemolyticus* ATCC 17802 **วิธีการศึกษา:** ทดสอบฤทธิ์ยับยั้งเชื้อแบคทีเรียโดยวิธี disc diffusion เพื่อหาบริเวณยับยั้งเชื้อ หาค่าความเข้มข้นต่ำสุดของสารสกัดที่สามารถยับยั้งเชื้อ (MIC) และหาค่าความเข้มข้นต่ำสุดของสารสกัดที่สามารถฆ่าเชื้อทั้ง 5 สายพันธุ์ (MBC) ด้วยวิธี broth micro dilution **ผลการศึกษา:** สารสกัดจากเปลือกต้นแคบ้านสามารถยับยั้งเชื้อแบคทีเรียก่อโรคอุจจาระร่วงที่นำมาศึกษาได้ทุกสายพันธุ์ ยกเว้น *S. enteritidis* DMST 15676 เมื่อทดสอบด้วยวิธี broth micro dilution สารสกัดจากเปลือกต้นแคบ้านยับยั้งเชื้อ *S. aureus* ATCC 25923 ได้ดีที่สุด โดยมีค่า MIC เท่ากับ 3.12 mg/ml และค่า MBC เท่ากับ 6.25 mg/ml **สรุป:** สารสกัดจากเปลือกต้นแคบ้านสามารถยับยั้งเชื้อแบคทีเรียก่อโรคอุจจาระร่วงบางสายพันธุ์ได้ ซึ่งสอดคล้องกับการใช้ประโยชน์ตามภูมิปัญญาพื้นบ้านของไทย

คำสำคัญ: ฤทธิ์ต้านเชื้อแบคทีเรีย, แบคทีเรียก่อโรคอุจจาระร่วง, สารสกัดจากเปลือกต้นแคบ้าน

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Abstract

Objective: To evaluate antibacterial activity of *S. grandiflora* (L.) Desv. bark extract on *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* DMST 15676, *Shigella sonnei* ATCC 11060 and *Vibrio parahaemolyticus* ATCC 17802. **Method:** Antibacterial activity tests were performed by using disc diffusion method to detect the inhibition zone, and micro broth dilution method to identify the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **Results:** The bark extract of *S. grandiflora* (L.) Desv. inhibited all bacterial strains except *S. enteritidis* DMST 15676. When tested with the broth micro-dilution technique, the extract inhibited *S. aureus* ATCC 25923 the most at the MIC of 3.12 mg/ml and the MBC of 6.25 mg/ml. **Conclusion:** These results indicated that the *S. grandiflora* (L.) Desv. bark extract is capable in inhibiting some diarrheal pathogens in accordance with Thai local wisdom.

Keywords: antibacterial activity, diarrheal pathogens, *Sesbania grandiflora* (L.) Desv. bark extract

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Introduction

Diarrhea is a common disease in the digestive system. Disease surveillance conducting from January to June 2017 revealed that 457,952 people from 77 provinces in Thailand had diarrhea (699.94 per 100,000 populations).[1] Since 2004 to 2013, the incidence varied from 50,000 to 120,000.[2] Diarrhea is one of the top-ten health problems in the public health system.

Patients normally confront the key symptoms of diarrhea including loose stool consistency, increased frequency, urgency of bowel movements, or incontinence.[3] Diarrhea can be caused by a range of pathogens including several bacteria. Most of them were aerobic bacteria such as *V. parahaemolyticus*, *S. sonnei*, S. enteridis, *E. coli* and *S. aureus*.[4,5]

Thai traditional medicine has long been recognized in Thai public health system for therapeutic purpose. Parts of herbs including fruit, leaves, root and have been used to treat various ailments including diarrhea. The use of ethnomedicine to relieve diarrhea has also been seen in other countries such as India, Nepal and Nigeria.[6-8] The Ninth National Research Policy and Strategy (2017-2021) of Thailand promoted the use and research of Thai herbs and alternative medicine for better healthcare service and cost containment. Many herbs have been used for therapeutic purpose regardless of the induction to the national essential drug list.

Sesbania grandiflora (L.) Desv. (Leguminosae family) has been widely used as food and medicine in Thailand. It was reported that medicinal products could be prepared from roots,

bark, gum, leaves, flowers and fruits of *S. grandiflora* in South-east Asia and India.[9] In Thai Traditional medicine, a bark decoction of *S. grandiflora* is taken orally to treat fever, gastric ailments, diarrhea, dysentery and diabetes. *S. grandiflora* has been reported to use as the main ingredient in the remedy to alleviate diarrhea.[10] A study of phytochemical screening of *S. grandiflora* in ethanol extract found tannins and gum to be rich in the bark.[11] Tannins, known to be antimicrobial biomolecules, could be found in various plants.[12] More studies are needed to confirm antimicrobial activity of *S. grandiflora*. This study aimed to investigate antibacterial activity of the bark extract of *S. grandiflora* (L.) Desv. against common diarrhea pathogens including *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. enteritidis* DMST 15676, *S. sonnei* ATCC 11060 and *V. parahaemolyticus* ATCC 17802.

Methods

Chemicals and devices

Absolute ethanol was from Fisher BioReagent™. Mueller-Hinton media with agar (MHA) and without agar (i.e., broth) (MHB) were from BD Difco™. For culture media, thiosulfate citrate bile salts sucrose agar (TCBS) and MacConkey agar (MAC) were from Oxoid™. Blood agar (BA) was prepared by sheep blood. Furthermore, tryticase soy broth (TSB) was from BD Difco™. Paper disc with a diameter of 6 mm from Whatman® was used.

Strains of bacteria and culture

Five strains of bacteria causing diarrhea including *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. enteritidis* DMST 15676, *S. sonnei* ATCC 11060 and *V. parahaemolyticus* ATCC 17802 were tested for antibacterial activity. The bacterial strains were provided from the culture collection of the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand.

S. aureus ATCC 25923 was subcultured on blood agar (BA) and *V. parahaemolyticus* ATCC 17802 on thiosulfate-citrate-bile salts-sucrose agar (TCBS agar). MacConkey agar was used to culture other bacterial strains. All strains were suspended in TSB and incubated at 37 °C for 24 hours. Turbidity was adjusted to match that of 0.5 McFarland standard to obtain a cell concentration of 10^7 CFU/ml.

Plant extraction

In this research, bark of *S. grandiflora* (L.) Desv. was collected from Sainoi district of Nonthaburi province, Thailand. The collected plant was identified based on plant taxonomy and morphology. Dark-brown bark of *S. grandiflora* (L.) Desv. was washed with water and then incubated at 50 °C till dry. Dry plant was grounded by blender and macerated with 95% ethanol based on the methods modified from the work of Khatun et al (2012)[13] with a ratio of dry sample to solvent of 1:5 for 3 days at room temperature. The extract was filtered and the solvent was evaporated by rotary evaporator and air dried at 40 °C. Crude extract was viscous with dark-brown color with a yield of crude extract of 10.35% w/w. Crude extracts was kept in amber colored glass container at 4 °C. Then, stock solutions of crude extracts were prepared by diluting the dried extracts with 95% ethanol solution to obtain a final concentration of 200 mg/ml and tested for antibacterial activity.

Disc diffusion assay

The test for antibacterial activity of *S. grandiflora* (L.) Desv. was modified from that of Huang, Xie and Gong (2000).[14] Bacterial cell was adjusted to a McFarland turbidity standard number of 0.5 and each bacterial suspension was swabbed on MHA plate. Then, filter paper discs of 10 microliters of the extract with a concentration of 200 mg/ml were aseptically placed on MHA medium. Each plate was incubated at 37 °C for 24 hours. The inhibition zone was observed and measured in millimeter of the zone diameter.

In all experiments, Ampicillin disc with a concentration of 10 µg/ml was used as a positive control and 95% ethanol as a negative control. All tests were carried out in triplicate.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Extract was further tested for MIC by broth microdilution modified from the method of Baron and Finegold (1990).[15] With a two-fold serial dilution by MHB in the 96-well microplate, the extract concentrations were ranged from 100 mg/ml to 0.781 mg/ml. The final volume of in each well was 200 microliters. The inoculum of each bacteria was adjusted to the concentration of 1 x 10^7 cfu/ml. A volume of 100 ml of inoculum suspensions was transferred to each well. The negative control was also performed using 95% ethanol.

Ampicillin final concentration ranged from 64 µg/ml to 0.13 µg/ml was used as a positive control drug. Triplicate wells were run for each concentration of the extracts. After incubation, lowest concentration that inhibited visible growth was recorded as the MIC.

To determine MBC, ten microliters of the exhibiting invisible growth suspension (from 96 well microplate) was spot to MHA plates. The plates were incubated at 37 °C for 24 hours. MBC was taken as the concentration of *S. grandiflora* (L.) Desv. extract that did not exhibit any bacterial growth on the media plates.

Results

Antibacterial activity based on disc diffusion method

S. grandiflora (L.) Desv. bark extract was investigated for their antibacterial activity against 5 strains of diarrheal pathogens including *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. enteritidis* DMST 15676, *S. sonnei* ATCC 11060 and *V. parahaemolyticus* ATCC 17802 using disc diffusion method. It was found that the extract inhibited the growth of microorganisms in all strains except *S. enteritidis* DMST 15676 (Table 1). The most inhibition effect of the extract was retarding microbial growth of *S. aureus* ATCC 25923 at a concentration of 200 mg/ml.

Table 1 Antibacterial activity of S. grandiflora (L.) Desv. extract by disc diffusion method presented as inhibition zone.

Bacterial strains	Inhibition zone diameter (mm)	
	Extract [200 mg/ml]	Ampicillin disc [10 µg/ml]
S. aureus ATCC 25923	15.5 ± 1.2	32.8 ± 0.6
E. coli ATCC 25922	8.0 ± 0.5	25.3 ± 1.0
S. enteritidis DMST 15676	N	28.8 ± 1.0
S. sonnei ATCC 11060	8.0 ± 0.5	25.5 ± 1.0
V. parahaemolyticus ATCC 17802	12.0 ± 1.0	24.7 ± 0.5

Note: N = no inhibition zone

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

All strains of bacteria with apparent inhibition zone from the disc diffusion assay were tested for MIC by micro-dilution method. It was found that the extract showed the highest activity against all 4 bacteria as shown in Table 2. The highest inhibitory activity of *S. grandiflora* (L.) Desv. bark extract was also found in *S. aureus* ATCC 25923 (MIC = 3.12 mg/ml) (Table 2).

The MBC was confirmed by the absence of bacterial growth of the exhibiting invisible growth suspension that was spot onto MHA plates. *S. grandiflora* (L.) Desv. bark extract showed potentially bactericidal activity against all bacterial strains except *E. coli* ATCC 25922 with MBC of >100 mg/ml. MBC of *S. grandiflora* (L.) Desv. bark extract against *S. aureus* ATCC 25923, *S. sonnei* ATCC 11060 and *V. parahaemolyticus* ATCC 17802 were 6.25, 100 and 50 mg/ml, respectively (Table 2).

Table 2 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of S. grandiflora (L.) Desv. Bark extract against bacterial pathogens by broth micro-dilution method.

Bacterial strains	Antibacterial activities of the extract	
	MIC (mg/ml)	MIC (mg/ml)
S. aureus ATCC 25923	3.12	6.25
E. coli ATCC 25922	100	> 100
S. sonnei ATCC 11060	50	100
V. parahaemolyticus ATCC 17802	25	50

Discussions and Conclusion

S. grandiflora (L.) Desv. bark has been used for treatment of diarrhea and dysentery in folk medicine.[16] This study found that bark extracts of *S. grandiflora* (L.) Desv. inhibited all diarrheal pathogens except *S. enteritidis* DMST 15676. The inhibition zone of the bark extracts of *S. grandiflora* (L.) Desv. against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. sonnei* ATCC 11060 and *V. parahaemolyticus* ATCC 17802 ranged from 8.0 to 15.5 mm. The inhibitory effect of the bark extract of *S. grandiflora* (L.) Desv. on bacteria was offered by tannin, a phenolic compound that was reported for inhibitory activity against bacterial species.[17]

In this study, *S. grandiflora* (L.) Desv. bark extract had strong antibacterial activity to gram positive bacteria (*S. aureus* ATCC 25923; MIC = 3.12 mg/ml) and active activity to some gram negative bacteria such as *V. parahaemolyticus* ATCC 17802 (MIC = 25 mg/ml) and *S. sonnei* ATCC 11060 (MIC = 50 mg/ml). *S. aureus* ATCC 25923 is known as clustering gram positive cocci bacterium that is very important in the process of the food product as it can be toxic in humans.[18] Gram positive bacteria cell walls are mostly composed of peptidoglycan layer (90%) and other coatings such as teichoic acid. On the other hand, Gram negative bacterial cell wall is layered with peptidoglycan for only 5 - 20% of cell wall, while the rest consists of protein,

lipopolysaccharide and lipoprotein.[19] The fundamental difference between Gram positive and negative bacteria cell wall components may cause different inhibitory effects of *S. grandiflora* (L.) Desv. bark extract against 5 strains of diarrheal pathogens.

Our findings were consistent with the study of Vipin et al (2011) revealing that *S. grandiflora* (L.) Desv. bark extract had inhibitory activity against *E. coli*.[20] The extract showed inhibition zone at 13.2 mm (stock solutions = 250 mg/ml) and MIC at 75.0 mg/ml. Moreover, Mueller et al (2015) found the effect of bark extract of *S. grandiflora* (L.) Desv. could inhibit *S. aureus* and *S. epidermidis* at MIC of 11.0 and 13.8 mg/ml, respectively.[21] MBC of the extract for both strains were 2.5 mg/ml. Similar to the study of antimicrobial activity of *Shorea roxburghii* G. Don against food pathogens, the result showed that the bark extract of *S. roxburghii* G. Don was more sensitive to gram positive bacteria than gram negative bacteria.[22] The taste of the bark of *S. grandiflora* (L.) Pers is as astringent as the bark of *S. roxburghii* and both have a large amount of tannins. Tannin compounds are water soluble and inhibit the growth of bacteria.[23] It is possible that the tannin substance was extracted in this study. As a result, the bark extracts inhibited the bacteria. However, Anantaworasakul et al (2011) reported bark extract of *S. grandiflora* (L.) Desv. could inhibit Gram positive bacteria better than Gram negative ones. The bark ethanolic extract of *S. grandiflora* (L.) Desv. could inhibit *S. aureus* (diameter of inhibition zone = 13.7 $\pm$ 0.6 mm) better than *E. coli* (diameter of inhibition zone = 9.4 $\pm$ 0.1 mm).[9]

Our results of antimicrobial activity of the *S. grandiflora* (L.) Desv. bark extract suggested that *S. enteritidis* DMST 15676 was the most resistant strain followed by *E. coli* ATCC 25922 and *S. sonnei* ATCC 11060. On the other hand, *S. aureus* ATCC 25923 and *V. parahaemolyticus* ATCC 17802 were the most susceptible strains to the extracted plants, respectively.

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