THE ANTIBACTERIAL ACTIVITY OF THE AQUEOUS EXTRACT OF *SIDA ACUTA* BURM. F.

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Abstract. Increasing bacterial resistance to antimicrobial drugs had led to the search for new antibacterial agents. A Thai medicinal plant, *Sida acuta* Burm. F. (SA), has been used for wound healing. The objective of this study was to determine the antibacterial activity of the aqueous extract of SA (SA-AE) against the antimicrobial-susceptible strains including three gram-positive bacteria: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *S. aureus* ATCC 29213; and four gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* DMST 8212 and *Pseudomonas aeruginosa* ATCC 27853, using the disc diffusion and broth microdilution methods. We also conducted high performance liquid chromatography (HPLC) profile on SA-AE to determine the major components. The results of disc diffusion test of SA-AE at 5 mg/disc showed no inhibition zone for all these strains. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were 16 mg/ml and 16 mg/ml, respectively, which were lower than MIC and MBC values of SA-AE for the other strains. HPLC reveals SA-AE contains para-hydroxybenzoic acid, ferulic acid and resveratrol. SA-AE should be explored further as an antimicrobial agent against *S. aureus*.

Keywords: *Sida acuta* Burm. F., antibacterial activity, *Staphylococcus aureus*

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INTRODUCTION

The incidence of multidrug resistant bacteria is increasing, leading to increasing mortality (McAdam *et al*, 2012). *Sida acuta* Burm. F. (SA) is a medicinal plant that has been used for wound healing (Mohideen *et al*, 2002). In India, Kenya and Nigeria, it has been used to treat fever, bronchitis, diarrhea, dysentery, malaria, gonorrhea, and to reduce inflammation

and treat skin diseases (Karou *et al*, 2007). Alkaloid from ethanolic extract of SA was found to inhibit growth of gram-positive bacteria, tested by broth microdilution assay, with minimal inhibitory concentration (MIC) values of 16-400 μg/ml and minimal bactericidal concentration (MBC) values ranging from 80 to 400 μg/ml (Karou *et al*, 2006). SA was reported to have antibacterial activity against clinical isolates of *S. aureus* (Iroha *et al*, 2009).

This study aimed to evaluate antibacterial activity of the aqueous extract of SA (SA-AE) against gram-positive and gram-negative bacteria. We also aimed to identify the components of SA-AE using high performance liquid chromatography (HPLC), and using gallic acid, chlorogenic acid, para-hydroxybensoic acid (*p*-HBA), ferulic acid, resveratrol and myricetin as standard compounds because they have antibacterial activity (Borges *et al*, 2013; Rajagopal and Agrawal, 2011; Paulo *et al*, 2011).

MATERIALS AND METHODS

Plant samples

Sida acuta Burm. F. (SA) samples were collected from Khon Kaen Province, Thailand during May 2014. The samples were identified by Prof Arunrat Chaveerach at Department of Biology, Faculty of Science, Khon Kaen University. The leaves of SA were washed and dried in a hot-air oven at 50ºC and then ground to fine powder using an electric blender. The powder was kept in air-tight container at room temperature until further analysis.

Aqueous extraction of *Sida acuta* Burm. F.

Fifty grams of SA fine powder was placed in 600 ml deionized water (DI) and boiled at 100ºC for 1 hour. It was then filtered through a cheesecloth and centrifuged at 2,000*g* for 10 minutes. The supernatant was then evaporated using a rotary evaporator at 40-60ºC. This product was what we used for the study. It was stored at room temperature in a desiccator with light protection until used. The percent yield of SA-AE was also calculated from fifty grams of the SA powder.

Bacterial strains

Three sensitive strains of gram-positive cocci (*E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213) and four strains of gram-negative bacilli (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. mirabilis* DMST 8212 and *P. aeruginosa* ATCC 27853) were used in this study.

Determination of antibacterial activity by the disc diffusion method

The disc diffusion method was performed following standard procedure (CLSI, 2015). Each bacterial strain was used to prepare a bacterial suspension of 0.5 McFarland in normal saline and streaked on Muller-Hinton agar (MHA) (Oxoid, Buckinghamshire, England). Paper discs (6 mm in diameter) were soaked with SA-AE at a concentration of 5 mg/disc. A trimethoprim-sulfamethoxazole (SXT; 25 μg) disc (Oxoid, Basingstoke, UK) was used as a control. The study and control discs were placed on MHA and incubated at 37ºC for 24 hours. The inhibition zones for the SA-AE and SXT discs for each bacterial strain were measured in millimeters.

Determination of antibacterial activity by broth microdilution method

The broth microdilution method was performed following the standard procedure of CLSI (2015). Seven bacterial suspensions of 0.5 McFarland were prepared and diluted to 1:100. SA-AE

Table 1
The antibacterial activity of *S. acuta* Burm. F. aqueous extract.

Bacterial strain	Diameter of inhibition zone in millimeter	
	SA-AE (5 mg/disc)	SXT (25 μg/disc)
E. faecalis ATCC 29212	6	27.50±0.70
S. aureus ATCC 25923	6	30.00±0.00
S. aureus ATCC 29213	6	29.00±1.40
E. coli ATCC 25922	6	26.25±1.05
K. pneumoniae ATCC 700603	6	12.50±0.70
P. mirabilis DMST 8212	6	6
P. aeruginosa ATCC 27853	6	6

SA-AE, *S. acuta* Burm. F. aqueous extract; SXT, trimethoprim-sulfamethoxazole.

and SXT (Sigma, Darmstadt, Germany) were prepared at concentrations in ranges of 0.125-32 mg/ml and 0.125-32 μg/ml, respectively. The 96-well plates were incubated at 37ºC for 24 hours.

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the samples that can inhibit visible bacterial growth (no turbidity in Muller-Hinton broth); this was determined using the broth microdilution method. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the sample that resulted in no gram-positive cocci growth on blood agar and gram-negative bacilli growth on MacConkey agar.

Determination of the components of *S. acuta* Burm. F. using high performance liquid chromatography (HPLC)

HPLC was performed following the method of a previous study (Palasap *et al*, 2014) with slightly modification. SA-AE and six standard phenolic compounds (Table 3) was each dissolved in 5% dimethyl sulfoxide (DMSO). Then 100 μl of each sample was passed through 0.20 μm particle size filter before injection in to a C18 reversed phase column (Luna C18; 15 cm x 3 mm; Phenomenex, Torrance, CA). Mobile phase were 100% methanol and 0.5% phosphoric acid at ratios of 5:95, 70:30, 90:10 and 5:95 at 0-17, 17-18, 18-20.50 and 20.5-25 min, respectively, with flow rate of 1 ml/minute. The eluted compounds were detected by using a UV-VIS detector Model 2489; Water, Milford, MA) at wavelength 270 nm. Peak areas of these compounds were compared with standard compounds.

Statistical analysis

The data were presented as mean and standard error of mean (mean±SE).

RESULTS

Antibacterial activity

Fifty grams of the SA powder yielded 11.6 g of SA-AE powder (23.2% yield). The antibacterial activity of the SA-AE and the control drug using the disc diffusion method are shown in Table 1. SA-AE at 5 mg/disc had no antibacterial activity. The MIC and MBC values of SA-AE against the studied bacteria are shown in Table 2. The MICs of the SA-AE were 16 and >32

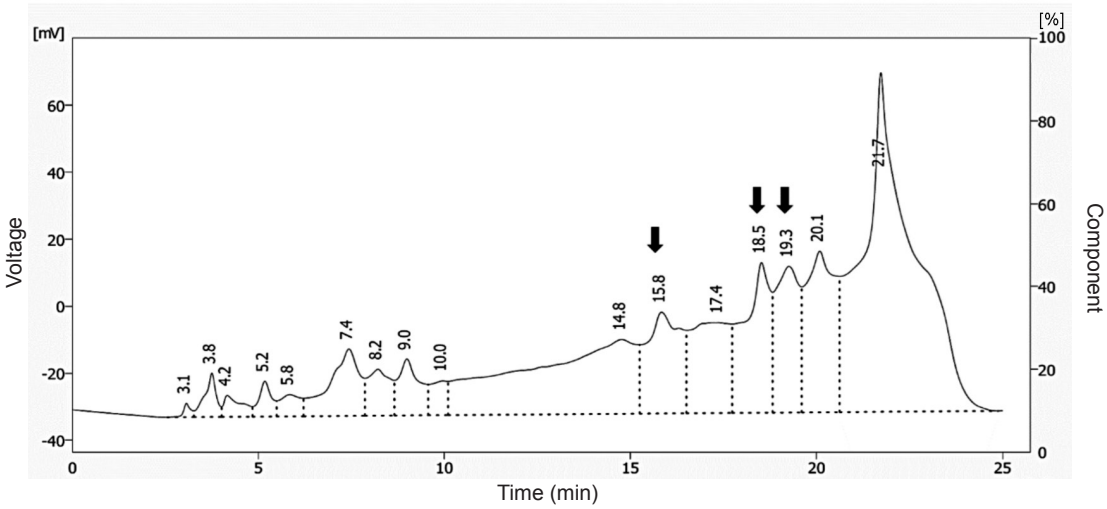

Fig 1–HPLC profile of *S. acuta* Burm. F. aqueous extract. Mobile phase: 100% methanol, 0.5% phosphoric acid. Flow rate: 1 ml/minute. Detection: Ultraviolet-visible detector at 270 nm. Retention time at 15.8, 18.5 and 19.3 minutes for *p*-hydroxybenzoic acid, ferulic acid and resveratrol, respectively.

mg/ml against the studied gram-positive cocci and gram-negative bacilli, respectively. The MBCs of the SA-AE were 16 mg/ml for both *S. aureus* ATCC 25923 and ATCC 29213 and the other strains had the MBCs of >32 mg/ml.

HPLC of SA-AE results are shown in Fig 1. Compared with the retention times of standard phenolic compounds (Table 3), SA-AE had peaks at the same retention time as HBA ferulic acid and resveratrol (retention times of 15.8, 18.5 and 19.3 minutes, respectively). The results, from calculation of area under peaks of SA-AE and standard phenolic compounds, showed that 10 μg of SA-AE may consist of 0.58 μg of *p*-HBA, 1.02 μg of ferulic acid and 0.62 μg of resveratrol.

DISCUSSION

SA is a plant that has been investigated for its antibacterial properties (Karou *et al*, 2006; 2007). It has been found to have phenolic and flavonoid compounds (Ekpo and Etim, 2009). Most of the plant phenolic compounds, for example, protocatechuic acids and betulinic acid from *Clusia burlemarxii* exhibited antibacterial activities against gram-positive bacteria (Ribeiro *et al*, 2011). In our present study, the SA-AE used was a crude extract. Therefore, the antibacterial activity may have been due to the synergistic effect of multiple components.

In our present study, the bacterial reference strains used were selected because they have good susceptibility to antibacterial agents (Jindal and Kumar, 2012; Reddy and Salunke, 2013). This may be the reason they are sensitive to the SA-AE. The SA-AE at a concentration of 5 mg/disc has no activity against the studied bacteria using the disc diffusion method. The amount of SA-AE (5 mg/disc) was 20 times higher than the standard drug

Table 2
Minimum inhibitory concentration and minimum bactericidal concentration of the aqueous extract of *S. acuta* Burm. F.

Bacterial strains	MIC		MBC	
	SA-AE (mg/ml)	SXT (μg/ml)	SA-AE (mg/ml)	SXT (μg/ml)
E. faecalis ATCC 29212	16.00	0.12/2.38	>32.00	0.25/4.75
S. aureus ATCC 25923	16.00	0.12/2.38	16.00	0.25/4.75
S. aureus ATCC 29213	16.00	0.12/2.38	16.00	0.25/4.75
E. coli ATCC 25922	>32.00	0.12/2.38	>32.00	0.12/2.38
K. pneumoniae ATCC 700603	>32.00	0.12/2.38	>32.00	0.12/2.38
P. mirabilis DMST 8212	>32.00	>16.00/304.00	>32.00	>16.00/304.00
P. aeruginosa ATCC 27853	>32.00	>16.00/304.00	>32.00	>16.00/304.00

SA-AE, *Sida acuta* Burm. F. aqueous extract; SXT, trimethoprim-sulfamethoxazole; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Table 3
The HPLC retention time of standard phenolic compounds and peak area and concentration of *S. acuta* Burm. F. aqueous extract.

SA-AE		Standard phenolic compounds used for comparison	Retention time in minutes
% peak area	Concentration of phytochemical compound (μg/ml)		
ND	ND	Gallic acid	9.8
ND	ND	Chlorogenic acid	15.0
6.69	0.58	p-Hydroxybenzoic acid	15.8
7.60	1.02	Ferulic acid	18.5
6.64	0.62	Resveratrol	19.3
ND	ND	Myricetin	20.8

HPLC, high performance liquid chromatography; SA-AE, *S. acute* Burm. F. aqueous extract.

SXT (25 μg/disc). This may be because SA-AE was the crude extract that may contain very low amounts of bioactive compounds that had antibacterial activity. A previous study found the extract of SA could inhibit gram-positive bacteria (*S. aureus* and *Bacillus subtilis*) growth but not gram-negative bacilli growth (*E. coli*, *K. pneumoniae* and *P. aeruginosa*) (Kumar *et al*, 2013). Since SA-AE has MIC and MBC values against *S. aureus* ATCC 25923 and ATCC 29213 at 16 mg/ml, this may be beneficial for the treatment of gram-positive bacterial infections, especially

S. aureus because of its increasing drug-resistance.

In our study, on HPLC of SA-AE, there were 3 peaks which could represent *p*-HBA, ferulic acid and resveratrol. Ferulic acid was reported to be an effective antibacterial agent against *E. coli* and *S. aureus* (Monte *et al*, 2014). *p*-HBA has been used as preservative agents (Rajagopal and Agrawal, 2011). These suggest possible component of SA-AE that could have efficacy against *S. aureus*.

In conclusion, SA-AE had antibacterial activity against both reference strains of *S. aureus* ATCC 25923 and 29213. Further studies are needed to identify the active components of SA-AE and determine their antibacterial efficacy against *S. aureus.*

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