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In vitro Antimicrobial Activity of *Schefflera leucantha*: The Potential of Respiratory Tract and Urinary Tract Infection Treatment

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Abstract: The antimicrobial activity testing to evaluated the possibility of *S. leucantha* for treatment of nosocomial infection such as respiratory tract and urinary tract infections. The aerial part of *S. leucantha* was extracted using aqueous system with yield of 0.7-1.3% of dried weight of dried plant's powder. The antibacterial activity of *S. leucantha* aqueous extract has been screened using agar diffusion method. The *S. leucantha* aqueous extract showed inhibitory effect on growth of *L. plantarum* ATCC 14917, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC 13315. The MICs of *S. leucantha* are in the rage of 8-16 g L^{-1} while MBCs are in the rage of 16-32 g L^{-1}. In conclusion, the aqueous extract of *S. leucantha* showed inhibitory effect on growth of respiratory tract and urinary tract infection bacteria at low concentration. This result may give supporting data of used *S. leucantha* as nosocomial infection treatment.

Key words: *S. leucantha* viguier, nosocomial infection, respiratory tract infection, urinary tract infection, anti-microbial, aqueous extract

INTRODUCTION

Patients with non-infectious disease who have to stay in hospital have high risk to acquire nosocomial infection (Asefzadeh, 2005). There are the reported about 10% of hospital patients will acquire an infection while stay in hospital (Asefzadeh, 2005). The common pathogenic bacteria such as *Escherichia coli*, *Klebsilla pneumoniae* (Saonuam *et al*., 2008), *Haemophilus influenza*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (Nicholls *et al*., 1975) have been reported worldwide. Therefore, the common nosocomial infections are respiratory tract (Nicholls *et al*., 1975) and urinary tract infections (Saonuam *et al*., 2008).

Medicinal plant has been prescribed in folk medicine for treating various diseases such as malignancy and infection diseases (Trakulsomboon *et al*., 2006). In tropical area are rich with plant for natural resources. However, it has been few reported about traditional medicine with scientific correlation. *Schefflera leucantha* Viguier has been used for cough relief long time ago in Thailand. It would be useful to evaluate the antimicrobial activity of *Schefflera leucantha* Viguier for nosocomial infection treatment.

Schefflera leucantha is the shrub belong to the family Araliaceae. It has been used as Thai and Chinese folklore for treatment of cold, allergies, asthma, chronic cough and respiratory tract infection (Potduang *et al*., 2007). The plant has been proving to possess various pharmacological activities such as hypoglycemic (Satayavivad *et al*., 1996) and insect repellant activities (Sinchaisri *et al*., 1988). It has been reported that saponin in this plant possess bronchodilator effect when tested on histamine and methacholine-induced bronchoconstriction in guinea pig trachea muscle (Witthawaskul *et al*., 2003). The plant aqueous and methanol extract have been reported no toxicity (acute and subacute toxicity tests) at dose of 5,000 and 1,000 mg kg^{-1} in rats (Witthawaskul *et al*., 2003). The study about antimicrobial activity of methanol extract has been reported by Potduang *et al*. (2007). However, the aqueous extract of this plant never been reported. This study has been investigated antimicrobial activity of *S. leucantha* aqueous extract against opportunistic bacteria causing respiratory tract and urinary tract infection and other pathogenic bacteria.

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MATERIALS AND METHODS

Plant collection and extraction: *Schefflera leucantha* viguier was collected and on May 2008 from Prachinburi Province, Thailand. The plant was identified by Department of Biology, Faculty of Science, Mahasarakham University, Thailand. Ten gram of dried aerial part of plant were boiled in 500 mL water and the residues of extraction were done the repeat of extraction 3 times. The yield of extraction was 0.7-1.3% of dried weight of dried plant's powder. In this study used pooled of batches of the same condition of extraction throughout the studies. The extraction and antimicrobial susceptibility tests were conducted on year 2008 at Department of Science, Mahasarakham University, Thailand.

Microorganisms: All of bacteria used in this study are American Type Culture Collection (ATCC). The gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917 and gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 10031, *Proteus vulgaris* ATCC 13315 and *Pseudomonas aeruginosa* ATCC 9721) bacteria, were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Antimicrobial sensitive test

Agar diffusion susceptibility test: The antibacterial activity of plant extract were screened using agar diffusion method as described in the standard guideline (Lorian, 1996). Briefly, bacteria cultures were grown in Tryptic soy agar at 37°C for 19 h and wash with sterile normal saline solution (NSS) (0.9% NaCl) to prepared standard McFarland No. 0.5 (10^8 colony-forming units (cfu)/mL. Twenty milliliter of Muller Hinton Agar (MHA) was poured in Petri disc and the bacteria suspension solution were swab in to the surface of culture medium by using sterile swabbing cotton. The sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) were place on the agar medium surface.

Different concentrations (1, 2 and 4 g L^{-1}) of plant solution were prepared using sterile water as vehicle and filled in to the stainless steel cylinders. After pre-diffusion at room temperature for 1 h, the plates were incubated at 37°C for 19 h. The 10 mg L^{-1} of gentamicin sulphate (Sigma Chemical Co., St. Louis, USA) solution was used as reference antibiotic.

MICs and MBCs determination using agar dilution and broth macro dilution: The MICs (Minimal Inhibitory Concentration) of plant extract was determined by using agar dilution method (Lorian, 1996), while MBCs (Minimal Bactericidal Concentration) were determined by using the broth macro-dilution method (Lorian,1996). Both methods were used gentamicin sulphate as reference antibiotic (Sigma Chemical Co., St. Louis, USA). The bacteria suspensions were prepared in normal saline solution at density adjusted to No. 0.5 McFarland turbidity. The inoculated bacterial cultures were incubated at 37°C. After 24 h of incubation the MICs were recorded by observed no spot of bacteria growth in agar dilution method, while no turbidity of broth culture tube in broth-macro dilution method. The MBCs was determined by no bacterial growth observed in sub culture of clear tube from broth-macro dilution test.

RESULTS

In this study, *S. leucantha* was extracted with aqueous system with of 0.7-1.3% of dried weight of dried plant's powder. The antimicrobial activity of the plant was assayed *in vitro* by agar diffusion, agar dilution and broth macro-dilution methods against 10 bacteria species.

The agar diffusion test revealed the inhibitory effect of the plant against *L. plantarum* ATCC 14917, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC 13315. The clear zone diameter are in the range of 12-16 mm at plant extract concentration 4 g L^{-1} (Table 1). Among tested bacteria *L. plantarum* is normal flora while *E.coli*, *K. pneumoniae* and *P. vulgaris* are nosocomial infection bacteria. The aqueous extract of the plant showed inhibitory effect against growth of *E. coli* and *K. pneumoniae* causing urinary tract infection while *P. vulgaris* is an opportunistic causing both of urinary tract infections and respiratory tract infections.

The Minimal Inhibitory Concentrations (MICs) of the plant was determined by using agar dilution method and confirmed by broth macro-dilution method while the Minimal Bactericidal Concentrations (MBCs) were determined using broth macro-dilution method. The MICs of *S. leucantha* are in the rage of 8-16 g L^{-1} while MBCs are in the rage of 16-32 g L^{-1} (Table 2). The most sensitive bacteria to the plant extract were gram negative bacteria than gram positive bacteria. This may because of the difference cell wall structure of the bacteria.

In conclusion, aqueous extract of *S. leucantha* showed inhibitory effect against respiratory tract and urinary tract infection bacteria at low concentrations. The results supported used of *S. leucantha* as nosocomial

Table 1: Inhibition zone diameters of *S. leucantha* aqueous extract against some nosocomial infection bacteria and normal flora bacteria

Bacteria	Gram	Inhibition zone diameter (mm)			Gentamicin sulphate (10 mgL^{-1})
		S. leucantha (4 g L^{-1})	*S. leucantha* (2 g L^{-1})	*S. leucantha* (1 g L^{-1})	
S. aureus ATCC 25923	+	nz	nz	nz	20.3±0.6
S. epidermidis ATCC 12228	+	nz	nz	nz	19.6±0.8
M. luteus ATCC 9341	+	nz	nz	nz	19.9±1.2
B. subtilis ATCC 6633	+	nz	nz	nz	20.2±1.8
L. plantarum ATCC 14917	+	16.1±0.3	nz	nz	19.7±0.9
E. coli ATCC 25922	-	14.6±0.7	nz	nz	20.2±1.0
K. pneumoniae ATCC 10031	-	12.1±1.3	nz	nz	20.5±0.7
S. typhimurium ATCC 14028	-	nz	nz	nz	17.9±1.8
Ps. aeruginosa ATCC 9721	-	nz	nz	nz	18.3±1.7
P. vulgaris ATCC 13315	-	12.3±0.4	nz	nz	19.9±1.3

nz: No inhibition zone; Data are expressed as Mean±SD

Table 2: The MICs and MBCs of *S. leucantha* aqueous extract against some nosocomial infection bacteria and normal flora bacteria

Bacteria	*S. leucantha* (g L^{-1})		Gentamicin sulphate (mg L^{-1})	
	MIC	MBC	MIC	MBC
L. plantarum ATCC 14917	8	16	<0.5	nd
E. coli ATCC 25922	16	32	<0.5	nd
K. pneumoniae ATCC 10031	16	32	<0.5	nd
P. vulgaris ATCC 13315	8	16	<0.5	nd

nd: Not determine

infection treatment. In addition, the toxicity test of this plant was need to indicated the safety doses of used this plant as remedy.

DISCUSSION

Recently, nosocomial infection bacteria decrease their susceptibility to antibiotic medicine and some developed to be multidrug resistant bacteria (Martinez and Baquero, 2002). Patients with none infection diseases who have to stay in hospital for long period such as heart disease, cancer disease and other chronic diseases have high risk to get nosocomial infection (Nicholls *et al*., 1975; Asefzadeh, 2005; Saonuam *et al*., 2008). Furthermore, in nurse and medical career person who need to contact with patient often as well. The difference structure or targets of attacking bacteria medicines were being used. However, treatment of multi-drug resistant bacteria consider complicated. It has been reported that some of bacteria were decreased its susceptibility rates to Tigecycline for 3 years follow-up study in USA (Dowzicky and Park, 2008).

The one candidate for antibacterial treatment is plant extract. The folk medicine treatment has been applied in many countries before antibiotics were existed. However, the scientific studies such biological activity and toxicology or even clinical studies of antibacterial activity of plant extracts still in few amounts. Therefore, the evaluation of antimicrobial activity of plant extract may give useful information.

In this study, antimicrobial activity of *S. leucantha* has been evaluated. The screening of antimicrobial activity of plant extract was conducted by agar diffusion method. The plant aqueous extract showed inhibition zone against *L. plantarum* ATCC 14917, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC 13315 (Table 1.) The *L. plantarum* is normal flora while *E. coli*, *K. pneumoniae* and *P. vulgaris* are nosocomial infection bacteria. In general, *E. coli* and *K. pneumoniae* causing urinary tract infection while *P. vulgaris* is an opportunistic causing both of urinary tract infections and respiratory tract infections.

The MIC of *S. leucantha* aqueous extract was determined using agar dilution method (and confirm with broth macro dilution method), while MBC was determined using broth macro dilution (0.5 -512 g L^{-1}). The MICs of plant aqueous extract were in the range of 8-16 g L^{-1} while the MBCs were in the range of 16-32 g L^{-1} (Table 2).

Previously, antibacterial of *S. leucantha* methanol extract has been reported by Potduang *et al*. (2007). The study reported antimicrobial activity of *S. leucantha* methanol extract against *Bacteroides* sp., *Enterococcus faecalis*, *Lactobacillus* sp., *Peptococcus* sp., *Streptococcus mutans*., *Klebsilla pneumoniae* and *Propionibacterium acnes*. However, the antimicrobial activity of plant extract can be difference due to the difference solvent extraction. The using of water considers less toxicity compare with organic solvent and good as medicine vehicle as well. In this study, *S. leucantha* aqueous extract show good antibacterial activity in low concentration. This may supporting the used of *S. leucantha* as a traditional medicine for nosocomial infection especially for respiratory tract and urinary tract infections.

In conclusion, the result showed good inhibitory effect of *S. leucantha* against respiratory tract and urinary tract infection bacteria at low concentration. It may interesting for further investigation in clinical isolation of the bacteria to evaluate the possible use of this plant as the remedy to treat respiratory tract and urinary tract infection because the bacteria that was isolated from patient may have difference susceptibility to the plant extract and also to the medicine as well. Recently, the infection diseases treatments with medicine have to deal with multi-drug resistant and adverse effect of the drug. The plant extract seem to be one way of treatment of infection disease. This study may supply some information of antimicrobial activity of *S. leucantha* aqueous extract against respiratory and urinary tract infection bacteria.

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