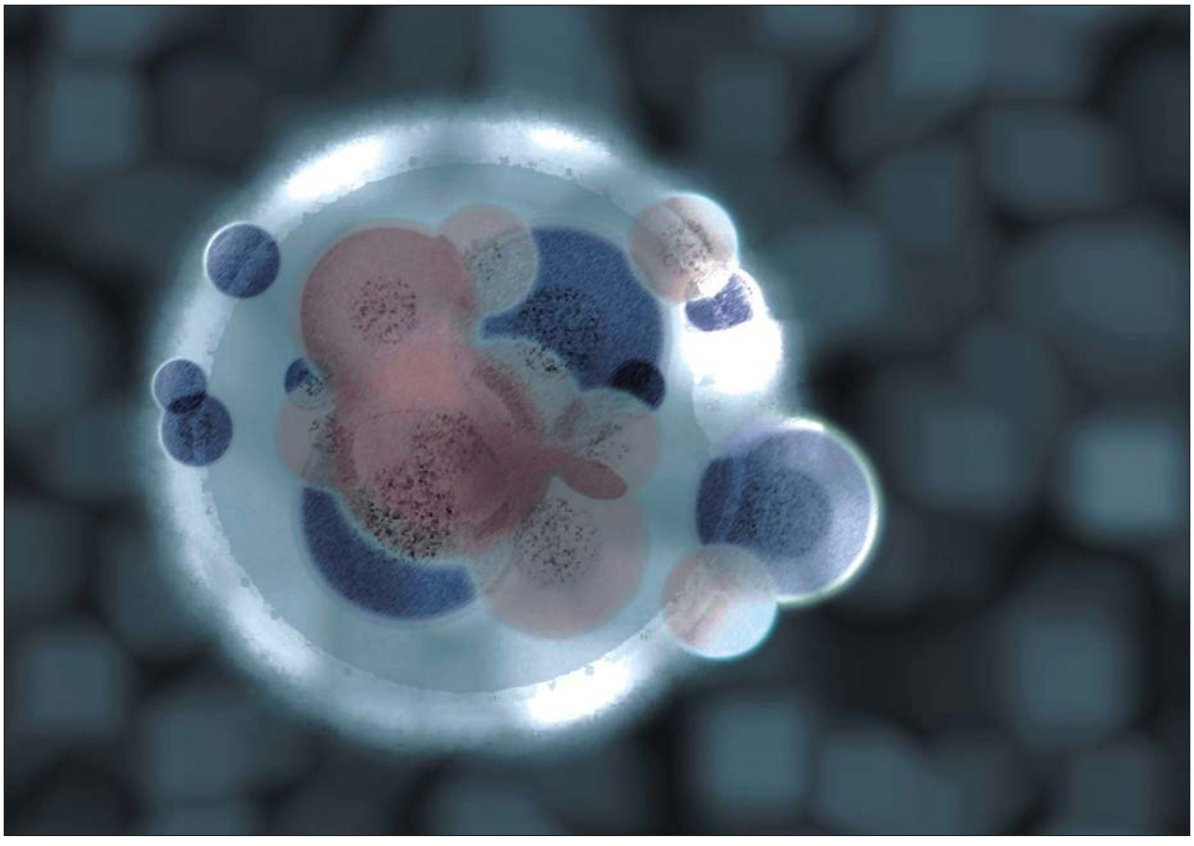

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Antimicrobial Properties of *Derris scandens* Aqueous Extract

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Abstract: The objective of this study is to evaluate the antimicrobial activity of *D. scandens* aqueous extract against various bacteria. The stems of *D. scandens* were extracted using aqueous extraction with yield 1.5-2% of dried weight of plant stems. The antimicrobial activity was screened by using agar diffusion method. Minimum Inhibitory Concentrations (MICs) and MBCs values were determined using agar dilution method and broth macro-dilution method. The agar diffusion tested revealed that the plant aqueous extract showed inhibition zone against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. coli* ATCC 25922. Among three bacteria, *S. aureus* and *S. epidermidis* is gram positive bacteria, while *E. coli* is gram negative bacteria. Minimum Bactericial Concentration (MICs) of the plant aqueous extract were in range of 2-4 g L^{-1}, while MBCs were in the range of 4-16 g L^{-1}. In conclusion, the plant extract showed good inhibitory effect on growth of *S. aureus* and *E. coli* which are nosocomial infection bacteria and those *S. epidermidis* which is normal flora.

Key words: *D. scandens*, leguminosae, aqueous extract, antimicrobial, nosocomial infection

INTRODUCTION

Nosocomial Infectious disease is a major problem in many health care systems. It has been reported that 10% of hospital patients will acquire an infection while in hospital (Asefzadeh, 2005). Infections can complicate illnesses, cause distress to patients and family and can in some cases lead to patient death. Among nosocomial infections, there are main infection that has been reported such as blood stream infections (28%), ventilator-associated pneumonia (21%), urinary tract infection (12%), lower respiratory infection (12%), gastrointestinal, skin, soft tissue and cardiovascular infections (10%), surgical-site infection (7%) and ear, nose and throat infection (7%) (Asefzadeh, 2005). The blood stream mostly causing by *Escherichia coli*, while urinary infection mostly causing by *Escherichia coli*, *Staphylococcus aureus* and *Krebsila pneumoniae* (Assefa *et al*., 2008; Maki *et al*., 2006; Laupland *et al*., 2008). It also has been reported that *E. coli*, *S. aureus* and *K. pneumoniae* decrease susceptibility to antibiotics treatment (Farooqi *et al*., 2000).

The nosocomial infection treatment is generally use antibiotics. However, there are reports about adverse effect of antibiotics (Lin *et al*., 2009; Khotaei *et al*., 2008). The adverse effect of antibiotic treatment sometime can be severe or even causing death (Lin *et al*., 2009). Furthermore, bacteria have genetically ability to transmit and acquire drug resistance (Nascimento, 2000). Thus, the antimicrobial activity of plant extract which considered came from natural products was became interesting.

In rain forest countries have abundance of plant as natural resources. Long time ago human learn to used natural product for maintain health as call folkloric medicine. The World Health Organization has supported to use medicinal plants as remedies (Nascimento, 2000). About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Nascimento, 2000). However, to used plants extract or chemical extract from plant as remedy need supporting data such as biological activities study, toxicological study etc. In this study has evaluated anti-microbial activity of *Derris scandens* which was used as Thai folkloric medicine.

Derris scandens Benth. (family Leguminosae) is a woody vine which has been used in Thai folkloric medicine (Sriwanthana and Chavalittumrong, 2001). In Thai traditional medicine, the stem of the plant has been used for expectorant, antitussive, diuretic, antidysentery and for treatment of cachexia (Tiangburanatham, 1996). The hypothesis of this study is that the polar compound from aqueous extract of *D. scandens* could show inhibitory effect on growth of selected nosocomial bacteria infection. The micro flora bacteria (*Lactobacillus plantarum*, *S. epidermidis*) were also included in this study. The aim of this study was to evaluate anti-microbial activity of *D. scandens* aqueous extract against 10 selected bacteria.

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MATERIALS AND METHODS

Plant material: Stems of *Derris scandens* Benth. were collected in June, 2006 from Singhaburi Province, Thailand. The plant was identified by botanist from Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand.

Extraction: Plant stems were dried in oven at 40°C for 1 day and grinded with blender. Ten grams of plant powder was boiled in 1 L of water for 15 min and filtrated. The residue of plant powder was repeated boiling procedure for 3 times and the filtrated were pooled and spry dried. The yield of spray dried extract was 1.5-2% of dried weight of plant stems.

Preparation of the samples: The spray dried powder was dissolved in sterile distilled water in concentration of 125, 250 and 500 g L^{-1}. The solution of gentamicin sulphate 10 mg L^{-1} (Sigma Chemical Co., St. Louis, USA) was used as reference antibiotic and 0.9% NaCl solution was used as negative control.

Microorganisms: Five strain of gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917) and five strains of gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Klebsiella pneumonia* ATCC 10031 *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9721) bacteria were used as test organisms.

Agar diffusion test: Antimicrobial testing of plant extract was conducted as standard guideline technique (Lorian, 1996). The solution of plant extract was prepared at concentration 125, 250 and 500 g L^{-1} using sterile distilled water. Bacterial suspension solutions were adjusted at turbidity of No. 0.5 standard McFarland (10^7 cfu mL^{-1}) in 0.9% NaCl solution. The bacteria suspensions were inoculated on plated containing Mueller-Hinton agar plate. Three hundred microliters solution of plant extract were filled in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) which place on the inoculated agar surface. The diameter of inhibition zone was measured after 19 h incubation at 37°C.

Agar dilution and broth macro-dilution methods: The Minimum Inhibitory Concentrations (MICs) were determined using agar dilution method and confirmed by result of broth macro-dilution method, while Minimum Bactericidal Concentration (MBCs) were determined using broth macro-dilution method according to standard guideline (Lorian, 1996). The agar dilution method was using plant extract concentration in range of 0.5-256 g L^{-1} in Mueller-Hinton agar and spot with 0.5 McFarland bacterial suspension. The MICs of the plant was recorded by observed no growth of bacteria on the agar surface at each concentration after incubated at 37°C for 24 h. While, broth macro-dilution method, plant solution were prepared in sterile water at concentration 256 g L^{-1} (range 0.5-256 g L^{-1}). Two fold serial dilutions in 3 mL of Mueller-Hinton broth were made and then 3 mL of bacterial suspension of was added to give final inoculums of 0.5×10^6 cfu mL^{-1}. The solutions were incubated at 37°C for 24 h. The MICs were recorded by observed the lowest concentration that showed no visible growth of bacteria while MBCs were recorded as the lowest concentration that showed no growth of bacteria after subculture on agar medium.

RESULTS AND DISCUSSION

The stems of *D. scandens* were e×tracted using aqueous system with yield 1.5-2% of dried weight of plant stems. The spray dried powder of the plant was dissolved in sterile water as solution for antimicrobial activity testing. The antimicrobial activity was screened by using agar diffusion method. MICs and MBCs values were determined using agar dilution method and broth macro-dilution method. All antimicrobial activity test methods were conducted using Mueller-Hinton medium and gentamicin sulphate as reference antibiotic.

The agar diffusion tested revealed that the plant aqueous extract showed inhibition zone against *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. coli* ATCC 25922 (Table 1). Among three bacteria, *S. aureus* and *S. epidermidis* is gram positive bacteria, while *E. coli* is gram negative bacteria. MICs of the plant aqueous extract were in range of 2-4 g L^{-1}, while MBCs were in the range of 4-16 g L^{-1} (Table 2). The plant extract showed good inhibitory effect on growth of *S. aureus* and *E. coli* which are nosocomial infection bacteria and those *S. epidermidis* which is normal flora bacteria as well. The results may support the data of antimicrobial activity of *D. scandens* aqueous extract. However, it may need toxicity and active compound studies to indicated safety of used this plant extract as remedy.

In Thailand, *Derris scandens* Benth. was used as traditional medicine for epectorant, antitussive, diuretic, antidysentery and for treatment of cacheia (Sriwanthana and Chavalittumrong, 2001). It chemical constituents have been reported are derrisisoflavones A-F (Sekine *et al*., 1999), eturunagarone, warangalone, 8-γ,γ-dimethylallyl-

Table 1: Inhibition zone diameters of *D. scandens* aqueous extract against various bacteria

Bacteria	Gram	Inhibition zone diameter of *D. scandens* aqueous extract (mm) 500	250	125	Gentamicin sulphate (10 mg L^{-1})
		($g L^{-1}$)			
S. aurens ATCC 25923	+	12.7+0.2	nz	nz	21.2±1.35
S. epidermidis ATCC 12228	+	16.4+0.3	12.9+0.6	nz	20.6±0.73
M. lutens ATCC 9341	+	nz	nz	nz	18.8±1.24
B. subtilis ATCC 6633	+	nz	nz	nz	19.4±1.32
L. plantarum ATCC 14917	+	nz	nz	nz	22.3±0.93
E. coli ATCC 25922	-	16.3+0.3	14.7+0.6	nz	22.1±1.22
K. pneumoniae ATCC 10031	-	nz	nz	nz	18.9±1.25
S. typhimurium ATCC 14028	-	nz	nz	nz	19.9±0.69
Ps. aeruginosa ATCC 9721	-	nz	nz	nz	19.8±1.96
P. vulgaris ATCC13315	-	nz	nz	nz	21.4±1.36

Data are mean±SD. n = 3. nz = No inhibition zone

Table 2: The MICs and MBCs of *D. scandens* aqueous extract against various bacteria

Bacteria	*D. scandens* aqueous extract MIC	*D. scandens* aqueous extract MBC	Gentamicin sulphate MIC	Gentamicin sulphate MBC
	($g L^{-1}$)		($mg L^{-1}$)	
S. aurens ATCC 25923	4	16	<0.5	nd
S. epidermidis ATCC 12228	2	4	<0.5	nd
E. coli ATCC 25922	4	8	<0.5	nd

nd: Not determine

wighteone, 3'-γ,γ-4,4'-di-O-methyl scandenin (Rao *et al*., 1994; Wang *et al*., 1997), scandenin, nallanin, chadanin (Clarke, 1943; Johnson *et al*., 1966), osajin, scandenone, scandinone (Pelter and Stainton, 1966), chandalone, lonchocarpic acid and ionchocarpenin (Johnson *et al*., 1966; Falshaw *et al*., 1969; Subba Rao and Seschadri, 1946; Mahabusarakam *et al*., 2004; Rukachaisirikul *et al*., 2002). It has been reported immunostimulating activity (Sriwanthana and Chavalittumrong, 2001), anti-oxidant and free radical scavenging activity (Rao, 2007), anti-inflammatory activity (Laupattarakasem *et al*., 2003, 2004). It never has been reported anti-microbial activity from this plant extract before.

The aim of this study was to evaluate the antimicrobial activity of *D. scandens* stem aqueous extract against 10 selected bacteria. There are 8 pathogenic bacteria (*S. aureus*, *E. coli*, *B. subtilis*, *M. luteus*, *K. pneumoniae*, *Ps. aeruginosa*, *S. typhimurium* and *P. vulgaris*) and 2 normal flora bacteria (*L. plantarum*, *S. epidermidis*). The hypothesis of this study is the *D. scandens* stem aqueous should show inhibitory effect against pathogenic bacteria, while not have effect on growth of normal flora bacteria. Among 8 pathogenic bacteria, *S. aureus*, *E. coli*, *K. pneumoniae*, *Ps. aeruginosa* and *P. vulgaris* are nosocomial infection bacteria (Saonuam *et al*., 2008; Laupland *et al*., 2008; Assefa *et al*., 2008; Farooqi *et al*., 2000). Mostly, the nosocomial infection bacteria are opportunistic bacteria which causing problem when human immune become weak from sickness (Saonuam *et al*., 2008).

The nosocomial infection that has been reported such as blood stream infections, ventilator-associated pneumonia, urinary tract infections, lower respiratory infections, gastrointestinal, skin, soft tissue and cardiovascular infections, surgical-site infections and ear, nose and throat infections can cause vital dangerous. It has been reported that *S. aureus* and *E. coli* causing serious nosocomial infection such as blood stream infections, urinary tract infections, skin infections and surgical-site infections (Farooqi *et al*., 2000; Asefzadeh, 2005; Assefa *et al*., 2008; Tillotson *et al*., 2008; Saonuam *et al*., 2008; Laupland *et al*., 2008). However, not only that *E. coli* and *S. aureus* are important nosocomial infection bacterial which cause many infection problem as mention above but also its susceptibility to antibiotic treatment has reported to be increase (Dowzicky and Park, 2008). Currently, using often antibiotic treatment showed the result of multi-drug resistant bacteria since the bacteria have ability to change the susceptible gene and become resistant to antibiotics. It has been reported that for about 50% of methicillin-resistant *S. aureus* (MRSA) are multidrug resistant and the number of it is more than doubled between 1999 and 2005 (Dowsicky and Park, 2008). While, β-lactamase producing *E. coli* has been reported in Thailand (Saonuam *et al*., 2008). This lead us to concern about the infection bacteria treatment especially nosocomial infection bacteria treatment should be in carefully way.

The problem of antibiotic treatment of nosocomial infection is not only about the bacteria itself. There are also reported about antibiotic treatment adverse effect in both children (Khotaei *et al*., 2008) and adult (Lin *et al*., 2009). The symptom of antibiotic adverse effect can be dermal allergic, gastrointestinal manifestations and/or even cause morbidity (Lin *et al*., 2009). Therefore, the applications of plant extract as antimicrobial therapeutic become interesting. This is parallel with World Health Organization (WHO) was supported the used of plant as remedy for local people.

The antimicrobial test of *D. scanden* aqueous extract showed inhibitory effect on growth of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. coli* ATCC 25922 at low concentration. Interestingly, the plant extract showed inhibitory effect against *S. aureus* and *E. coli* which are nosocomial infection bacteria and those normal flora bacteria *S. epidermidis*. Even thought the aqueous extract of *D. scanden* showed the inhibitory effect on growth of *S. epidermidis* which is normal flora it also showed inhibitory effect against *S. aureus* and *E. coli* which is causing many nosocomial infections at low concentration. In conclusion, aqueous extract of *D. scandens* showed good inhibitory effect on growth of nosocomial infections bacteria *S. aureus* and *E. coli* and normal flora bacteria *S. epidermidis* at low concentration. This is a first report of antimicrobial activity of *D. scandens* aqueous extract and additional data for biological activity of its aqueous extract. This also may supported the used of *D. scandens* as antibacterial remedy for nosocomial infections disease.

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