



International Journal of **Biological Chemistry**

ISSN 1819-155X



Academic
Journals Inc.

www.academicjournals.com

Antimicrobial Activity of *Acanthus ebracteatus* Vahl. Aqueous Extract: The Potential for Skin Infection Treatment

¹C. Sittiwet, ²N. Niamsa and ¹D. Puangpronpitag

¹Biomedical Research Unit,

²Polymer Science and Technology Research Unit, Department of Chemistry,
Faculty of Science, Mahasarakham University, Khamreung,
Kantharawichai, Mahasarakham 44150, Thailand

Abstract: The *A. ebracteatus* was extracted in boiling water with 0.7-1.3% yields. The antimicrobial activity of *A. ebracteatus* aqueous extract has been screened using agar diffusion method. *A. ebracteatus* aqueous extract showed inhibitory effect on growth of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *L. plantarum* ATCC 14917, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC13315. The MICs and MBCs of *A. ebracteatus* has been evaluated using agar dilution and broth macro dilution methods. The MICs and MBCs of *A. ebracteatus* aqueous extract are in the range of 1-2 and 2-4 g L⁻¹, respectively. In conclusion, *A. ebracteatus* aqueous extract showed good antimicrobial activity against nosocomial pathogen and skin infection bacteria at low concentrations. This might supported the used of *A. ebracteatus* to treat nosocomial infection and skin infections.

Key words: *A. ebracteatus*, skin infection, skin infection bacteria, anti-microbial, aqueous extract

INTRODUCTION

Nosocomial infections are the main problem for patients who need to admit in hospital for some period. There are major of nosocomial infections such as respiratory tract, urinary tract, surgical wound, blood-stream and skin and soft tissue infections (Saonuam *et al.*, 2008). The skin and soft tissue infections mostly caused by *Staphylococcus aureus* and *Streptococcus* sp. (Stulberg *et al.*, 2002) especially *S. aureus* tend to decrease its susceptibility to the antibiotic and become multi-drug resistant bacteria (Tillotson *et al.*, 2008; Dowzicky and Park, 2008).

In general, the ways to treat nosocomial infections are to use antibiotic. However, the antibiotic treatments also give vital of severe adverse effects in patients (Khotaei *et al.*, 2008). Therefore, the antibacterial activity of plant extract has been interested. The plant extract came from natural origin and has been used as remedy before the chemical medicines were used. The study about antibacterial activity of plant extract may give useful information to use plant extract as a remedy.

Acanthus ebracteatus Vahl. is a mangrove plant distributed in Southeast Asia. The plant has been used as folkloric medicine in Malaysia, Thailand and China for treatment of cough, hepatosplenomegaly, hepatitis, lymphoma, asthma, longevity, skin diseases, inflammatory and arthritis (Hokputsa *et al.*, 2004; Laupattarakasem *et al.*, 2003). The plant has been evaluated the content of megastigmane, aliphatic alcohol and benzoxazinoid glycoside (Kanchanapoom *et al.*, 2001c) and also has been compared within genus *Acanthus* as well (Kanchanapoom *et al.*, 2001a, b). In this study, the antibacterial activity of *A. ebracteatus* aqueous extract has been tested against various bacteria to evaluate the possibility of using it as skin infection remedy.

Corresponding Author: Chaityasit Sittiwet, Biomedical Research Unit, Department of Chemistry, Faculty of Science, Mahasarakham University, Khamreung, Kantharawichai, Mahasarakham 44150, Thailand
Tel: +6640170171 Fax: +6643754246

MATERIALS AND METHODS

Plant Sample and Extraction

A. ebracteatus was collected in Pattani Province, Thailand on May 2008. The identification of the plant was carried out by Department of Biology, Faculty of Science, Mahasarakham University, Thailand. The extraction was carried out by boiling 10 g of sample plant in 500 mL water. The aqueous extracts were filtered and spray dried in to powder. The yield of extraction was 0.7-1.3% of dried weight of dried plant's aerial part.

Tested Microorganisms

The tested microorganisms used in this study were as follows:

- **Gram positive bacteria:** *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633 and *Lactobacillus plantarum* ATCC 14917
- **Gram negative bacteria:** *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 10031, *Proteus vulgaris* ATCC 13315 and *Pseudomonas aeruginosa* ATCC 9721

All bacteria were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. Bacteria were inoculated on Tryptic Soy Agar (TSA) for 19 h at 37°C and washed from the agar surface with sterile Normal Saline Solution (NSS) (0.9% NaCl). Then adjusted the turbidity of bacterial suspension to a standard McFarland No. 0.5 (10^8 colony-forming units (cfu mL⁻¹)) before used as the starter solution.

Antimicrobial Susceptibility Test

Agar Diffusion Susceptibility Test

Agar diffusion susceptibility determinations were made as described in the standard guideline technique (Lorian, 1996). Twenty milliliter of Mueller Hinton Agar (MHA) was put in cultivation plates and swabbed with starter solution on the agar surface, by using swab cotton.

Spray dried powder of *A. ebracteatus* aqueous extract was dissolved in sterile water and put in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) that were placed on the inoculated agar surface. Different concentrations of plant extract solution were filled in the cylinders (300 µL cylinder⁻¹). After pre-diffusion at room temperature for 1 h, the plates were incubated at 37°C for 19 h. The NSS filled in the cylinder was used as control and a 10 mg L⁻¹ gentamicin sulphate (Sigma Chemical Co., St. Louis, USA) solution was used as standard in same cultivation plate.

MICs and MBCs Determination Using Agar Dilution and Broth Macro Dilution Methods

MICs of *A. ebracteatus* aqueous extract was determined by the agar dilution method (Merck) (Lorian, 1996) while MBCs were determined by the broth macro-dilution method (Lorian, 1996) and using gentamicin sulphate as reference antibiotics (Sigma Chemical Co., St. Louis, USA). Inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard (10^8 colony-forming units (cfu mL⁻¹)) and two fold dilution for the broth macro-dilution procedure. The inoculated tubes were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of plant extract or gentamicin sulphate at which the microorganism tested did not showed visible growth, while MBC was defined as the minimum bactericidal concentration with negative subcultures on the agar medium. Values were means of three measurements.

RESULTS AND DISCUSSION

A. ebracteatus has been used for anti-inflammation and skin disease treatment (Hokputsa, 2004). It has been reported anti-inflammatory effect (Laupattarakasem *et al.*, 2003) which is very good supporting evidence for using *A. ebracteatus* as skin disease treatment remedy. This study has been evaluated antibacterial activity of *A. ebracteatus* aqueous extract against various bacteria include *S. aureus* which is common skin infection bacteria.

The *A. ebracteatus* aqueous extract showed inhibitory effect on growth of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *L. plantarum* ATCC 14917, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC13315 (Table 1). The MICs and MBCs of *A. ebracteatus* aqueous extract are in the range of 1-2 and 2-4 g L⁻¹, respectively (Table 2). The result showed the antimicrobial activity of *A. ebracteatus* aqueous extract against skin infection bacteria (*S. aureus* ATCC25923), urinary and respiratory tract infections (*K. pneumoniae* ATCC10031 and *P. vulgaris* ATCC13315) and normal flora (*S. epidermidis* ATCC12228 and *L. plantarum* ATCC14917). The most pathogenic bacteria that the plant extract can inhibit are nosocomial infection bacteria. In conclusion, *A. ebracteatus* aqueous extract showed good antimicrobial activity against nosocomial pathogen bacteria at low concentration. It should support to use the plant extract as skin infection treatment and also for other nosocomial infection such as urinary tract and respiratory tract infections.

Nosocomial skin infection has been reported about 5.66% of nosocomial infections in Thailand (Saonua *et al.*, 2008; Asefzadeh, 2005). The common skin infection bacteria are *S. aureus* and *Streptococcus* sp. (Stulberg *et al.*, 2002). These groups of bacteria also decrease its susceptibility to antibiotic treatment and can become Multi Drug Resistance (MRD) bacteria which cause vital infections (Tillotson *et al.*, 2008; Dowzicky and Park, 2008). One reason causing MRD is long term treatment of antibiotic in patients especially HIV patients. The other problem with antibiotic medicine is the adverse effect. It has been reported that antibiotic using in children showed 12% of adverse effect in Iran (Khotaei *et al.*, 2008). The application of plants extract as remedy has become interesting by this reasons.

Table 1: Inhibition zone diameters of *A. ebracteatus* aqueous extract against various bacteria

Bacterias	Gram	Inhibition zone diameter of <i>A. ebracteatus</i> aqueous extract (mm)			Gentamicin sulphate (10 mg L ⁻¹)
		500 g L ⁻¹	250 g L ⁻¹	125 g L ⁻¹	
<i>S. aureus</i> ATCC 25923	+	16.9±1.3	12.5±0.9	nz	20.0±2.65
<i>S. epidermidis</i> ATCC 12228	+	17.8±0.7	nz	nz	20.7±1.52
<i>M. luteus</i> ATCC 9341	+	nz	nz	nz	19.3±2.51
<i>B. subtilis</i> ATCC 6633	+	nz	nz	nz	19.3±0.57
<i>L. plantarum</i> ATCC 14917	+	22.3±0.9	17.6±0.7	nz	20.3±0.52
<i>E. coli</i> ATCC 25922	-	nz	nz	nz	21.6±0.57
<i>K. pneumoniae</i> ATCC 10031	-	28.0±0.6	15.6±0.9	nz	17.6±1.53
<i>S. typhimurium</i> ATCC 14028	-	nz	nz	nz	18.0±1.00
<i>Ps. aeruginosa</i> ATCC 9721	-	nz	nz	nz	19.6±0.32
<i>P. vulgaris</i> ATCC13315	-	17.3±0.8	11.3±1.3	nz	20.0±0.46

Data are expressed as Mean±SD (n = 3); nz : No inhibition zone

Table 2: The MICs and MBCs of *A. ebracteatus* aqueous extract against various bacteria

Bacterias	<i>A. ebracteatus</i> aqueous extract (g L ⁻¹)		Gentamicin sulphate (mg L ⁻¹)	
	MIC	MBC	MIC	MBC
<i>S. aureus</i> ATCC 25923	2	4	<0.5	nd
<i>S. epidermidis</i> ATCC 12228	2	4	<0.5	nd
<i>L. plantarum</i> ATCC 14917	2	4	<0.5	nd
<i>K. pneumoniae</i> ATCC 10031	1	2	<0.5	nd
<i>P. vulgaris</i> ATCC 13315	1	2	<0.5	nd

nd: Not determine

In this study, *A. ebracteatus* aqueous extract has been tested against nosocomial especially skin infection bacteria. It was found that the plant extract showed inhibitory effect on growth of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *L. plantarum* ATCC 14917, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC13315 at low concentration of MICs and MBCs. Previously, the *A. ebracteatus* was reported immunomodulatory (Hokputsa *et al.*, 2004) and anti-inflammatory activities (Laupattarakasem *et al.*, 2003). It also was reported that *A. ebracteatus* contained megastigmane, benzoxazinoid glycosides and aliphatic alcohol (Kanchanapoom *et al.*, 2001b).

According to the plant has been used for treating skin diseases in Thailand the investigation of anti-microbial activity of this plant was to find out the possibility to used this plant for skin infection treatment. In earlier study, showed the immune stimulating effect of this plant which give high potential to used this plant for treatment of bacterial infection. In addition, the results from this study also supported that *A. ebracteatus* showed anti-microbial activity against tested skin infection bacteria and other nosocomial infections. This might supported the used of *A. ebracteatus* as skin infection and nosocomial infection treatment.

REFERENCES

- Asefzadeh, M., 2005. The risk of nosocomial infections for NCD patients. NCD Malaysia, 4: 8-12.
- Dowzicky, M.J. and C.H. Park, 2008. Update on antimicrobial susceptibility rates among gram-negative and gram-positive organisms in the United States: Results from the tigecycline evaluation and surveillance trial (TEST) 2005 to 2007. Clin. Ther., 30: 2040-2050.
- Hokputsa, S., S.E. Harding, K. Inngjerdingen, K. Jumel and T.E. Michaelson *et al.*, 2004. Bioactive polysaccharides from the stems of the Thai medicinal plant *Acanthus ebracteatus*: Their chemical and physical features. Carbohydrate Res., 339: 753-762.
- Kanchanapoom, T., M.S. Kamel, K. Kasai, K. Yamasaki, C. Picheansoonthorn and Y. Hiraga, 2001a. Lignan glucosides from *Acanthus ilicifolius*. Phytochemistry, 56: 369-372.
- Kanchanapoom, T., M.S. Kamel, K. Kasai, C. Picheansoonthorn, Y. Hiraga and K. Yamasaki, 2001b. Benzoxazinoid glucosides from *Acanthus ilicifolius*. Phytochemistry, 58: 637-640.
- Kanchanapoom, T., R. Kasai, C. Picheansoonthorn and K. Yamasaki, 2001c. Megastigmane, aliphatic alcohol and benzoxazinoids glycosides from *Acanthus ebracteatus*. Phytochemistry, 58: 811-817.
- Khotaei, G.T., F. Fattahi, Z. Pourpak, Z. Moinfar, F.M. Aghaei, K. Gholami and M. Moin, 2008. Adverse reactions to antibiotics in hospitalized Iranian children. J. Microbiol. Immunol. Infect., 41: 160-164.
- Laupattarakasem, P., P.J. Houghton, J.R.S. Hoult and A. Itharat, 2003. An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. J. Ethnopharmacol., 85: 207-215.
- Lorian, V., 1996. Antibiotics in Laboratory Medicine. 4th Edn., Williams and Wilkins, Baltimore, London, ISBN: 9780781749831.
- Saonum, P., N. Hiransuthikul, C. Suankratay, K. Malathum and S. Danchaivijitr, 2008. Risk factors for nosocomial infections caused by extended-spectrum β -lactamase producing *Escherichia coli* or *Klebsilla pneumoniae* in Thailand. Asian Biomed., 2: 485-491.
- Stulberg, D.L., M.A. Penrod and R.A. Blatny, 2002. Common bacterial skin infections. Am. Fam. Physician, 66: 119-124.
- Tillotson, G.S., D.C. Draghi, D.F. Sahm, K.M. Tomfohrde, T. del Fabro and I.A. Critchley, 2008. Susceptibility of *Staphylococcus aureus* isolated from skin and wound infections in the United States 2005-07: Laboratory-based surveillance study. J. Antimicrob. Chemother., 62: 109-115.