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***In vitro* Antimicrobial Activity of *Pluchea indica* Aqueous Extract: The Potential for Urinary Tract Infection Treatment**

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Abstract: The *P. indica* aqueous extract was tested against both gram positive bacteria (*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *M. luteus* ATCC 9341, *B. subtilis* ATCC 6633 and *L. plantarum* ATCC 14917) and gram negative (*E. coli* ATCC25922, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315, *Ps. aeruginosa* ATCC 9721) using agar diffusion susceptibility test. The result showed zone of inhibition against *E. coli* and *K. pneumoniae*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are between 1-2 and 4-8 mg L⁻¹ respectively. This result show the possibility of using *P. indica* as an alternative therapy in the treatment of urinary tract infections.

Key words: *P. indica*, nosocomial infection, urinary tract infection, anti-microbial, susceptibility test

INTRODUCTION

The *Escherichia coli* species of bacteria are the most commonest, isolated from patients with urinary tract infections (Nicolle *et al.*, 2005). There are also other gram negative bacteria isolated from urinary tract infection complication such as *Klebsilla pneumoniae*, *Proteus mirabilis*, *Providencia* species, *Pseudomonas aeruginosa* and other gram negative and gram positive bacteria mention (Nicolle *et al.*, 2005).

In Thailand urinary tract infection is one of the nosocomial infection (Danchaivijitr *et al.*, 2007). Among nosocomial infection in Thailand, urinary tract infection was estimated at 25% (Danchaivijitr *et al.*, 2007). Recently, the rate of bacterial antibiotic resistance is increasing (Chomvarin *et al.*, 2005; Pop-Vicas *et al.*, 2008). The use of plant extracts and phytochemicals, with known antimicrobial properties can be of great significance in chemotherapeutic treatment. *P. indica* belong to the family of Asteraceae has been reported biological activity such as anti-inflammatory (Sen and Nag Chaudhuri, 1991), anti-ulcer (Sen *et al.*, 1993), diuretic (Pramnik *et al.*, 2006), neutralized sneek and anti-amoebic activities (Biswas *et al.*, 2007).

The aim of this study is therefore to investigate the antimicrobial activity of the aqueous extract of the aerial part *P. indica* against both gram positive and gram negative bacteria frequently found in urinary tract infections.

MATERIALS AND METHODS

Sample Collection, Identification and Preparation of Extract

The *Pluchea indica* was collected and identified by Department of Biology, Faculty of Science, Mahasarakham University on May 2008 from Singhaburi Province, Thailand. The 10 g of dried plant was grinded and boil in 1 L of water for 15 min and filtrated was spray dried. The yield of spray dried extract was 10-18% of dried weight of dried plant. In this study used one batch of extraction throughout the studies.

Microbial Cultures

Laboratory isolates of the pure culture of gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917 and gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 10031, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9721) bacteria, were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Antimicrobial Test

Agar Diffusion Susceptibility Test

Disc diffusion agar gel method was made as described in the standard guideline technique (Lorian, 1996). All test bacteria were cultured overnight on Tryptic Soy Agar (TSA) slant at 37°C. Bacteria were washed from surface agar slant with sterile Normal Saline Solution (NSS) (0.9% NaCl) then adjusted to match turbidity of standard Mcfarland No. 0.5 before used as starter solution. Twenty milliliter of Mueller Hinton Agar (MHA) was put in cultivation plates and swabbed starter solution on agar surface by using swab cotton.

Pluchea indica aqueous extract was dissolved in sterile water and put in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the inoculated agar surface. The various 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 10 mg L⁻¹ gentamicin sulphate solution was used as standard in same cultivation plate.

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

MICs of plant extract were determined by agar dilution method while MBCs were determined by broth macro-dilution method (Lorian, 1996). The concentration of both plant extract and gentamicin sulphate were prepared in range of 0.5-512 mg L⁻¹ in Mueller Hinton Broth (MHB). Inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard (10⁸ colony-forming units (cfu mL⁻¹) and two fold dilution for the broth macro-dilution procedure. The inoculated tube were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was recorded as the lowest concentration of ME or gentamicin sulphate that completely inhibited the growth of the test organism while MBC was recorded as the minimum bactericidal concentration with negative subcultures on agar medium. Values were means of triplicate.

RESULTS AND DISCUSSION

The urinary tract infection is the one of nosocomial infections. The major bacteria cause urinary tract infection are *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Providencia* species and *Ps. aeruginosa* (Table 1). Urinary tract infection occur both in both adults and children. The prevalence of urinary tract infection has been reported in Thailand (Danchaivijitr *et al.*, 2007), north India (Habibi *et al.*, 2008), Italy (Hewitt *et al.*, 2008) and other countries. In this study, the antimicrobial activity of *Pluchea indica* was investigated and found to have potential against bacterial infections.

Pluchea indica is an important medicinal plant in oriental folklore medicine, especially in Thailand. It has been reported to have anti-amoebic activities against *Entamoeba* (Biswas *et al.*, 2007), anti-inflammatory (Sen and Nag Chaudhuri, 1991), anti-ulcer (Sen *et al.*, 1993), antioxidant (Sen *et al.*, 2002) and diuretic activities (Prannik *et al.*, 2006).

In this study *P. indica* aqueous extract has been tested against both of gram positive and gram negative bacteria (*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *M. luteus* ATCC 9341,

Table 1: Inhibition zone diameters of *P. indica* aqueous extract solution against various bacteria

Bacteria	Gram	Inhibition zone diameter (mm)			Gentamicin sulphate (10 µg mL ⁻¹)
		<i>P. indica</i> (100 mg mL ⁻¹)	<i>P. indica</i> (50 mg mL ⁻¹)	<i>P. indica</i> (25 mg mL ⁻¹)	
<i>S. aureus</i> ATCC 25923	+	nz	nz	nz	21.0±2.65
<i>S. epidermidis</i> ATCC 12228	+	nz	nz	nz	21.7±1.52
<i>M. luteus</i> ATCC 9341	+	nz	nz	nz	20.3±2.51
<i>B. subtilis</i> ATCC 6633	+	nz	nz	nz	18.3±0.57
<i>L. plantarum</i> ATCC 14917	+	nz	nz	nz	23.3±0.57
<i>E. coli</i> ATCC 25922	-	21.9	17.3	14.6	21.6±0.57
<i>K. pneumoniae</i> ATCC 10031	-	18.6	11.7±0.6	nz	17.6±1.53
<i>S. typhimurium</i> ATCC 14028	-	nz	nz	nz	18.0±1.0
<i>Ps. aeruginosa</i> ATCC 9721	-	nz	nz	nz	19.6±0.32
<i>P. vulgaris</i> ATCC13315	-	nz	nz	nz	20.0±0.46

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

Table 2: The MICs and MBCs of *P. indica* aqueous extract against various bacteria

Bacteria	<i>P. indica</i>		Gentamicin sulphate	
	MIC (mg L ⁻¹)	MBC (mg L ⁻¹)	MIC (mg L ⁻¹)	MBC (mg L ⁻¹)
<i>E. coli</i> ATCC 25922	1	4	<0.5	nd
<i>K. pneumoniae</i> ATCC 10031	2	8	<0.5	nd

nd: Not determine

B. subtilis ATCC 6633 and *L. plantarum* ATCC 14917) and gram negative (*E. coli* ATCC25922, *S. typhimurium* ATCC 14028 *K. pneumoniae* ATCC 10031 *P. vulgaris* ATCC 13315, *Ps. aeruginosa* ATCC 9721) using agar diffusion susceptibility (Table 2). It show inhibition zone against *E. coli* and *K. pneumoniae*. The further investigation of MICs are 1-2 mg L⁻¹ and MBCs are 4-8 mg L⁻¹, respectively. This result might give the possibility that *P. indica* may used as the urinary tract remedy.

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