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Antimicrobial Activity of Essential Oil from *Nelumbo nucifera* Gaertn. Pollen

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Abstract: The *Nelumbo nucifera* Gaertn. pollen essential oil was extracted by using vapor distillation. Antimicrobial activity of *Nelumbo nucifera* Gaertn. pollen essential oil has been investigated using agar diffusion susceptibility test and broth macro-dilution. The essential oil were tested against both gram positive (*S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *M. luteus* ATCC 9341, *B. subtillis* 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) bacteria using agar diffusion susceptibility test. Significant zone of inhibition were observed for *S. typhimurium* ATCC14028 and *E. coli* ATCC25922). The MICs and MBCs are 10-40 and 20-80 ml L^{-1}, respectively. *Nelumbo nucifera* pollen essential oil show inhibitory effect on growth of food born pathogen bacteria in low concentration which indicated the possibility of used as food preservation additive.

Key words: *Nelumbo nucifera*, food borne pathogen, anti-microbial, Nymphaceae

INTRODUCTION

The *Nelumbo nucifera* Gaertn. is an aquatic plant belonging to the family Nymphaceae (Gaertn.) which almost all parts are edible (La-ongsri *et al*., 2008). The leaves, root and the embryonic stage of the plant have reported to contain alkaloids such as such as roemerine, nuciferine, nornuciferine, nelumboside, anonaine, 5-methoxy-6-hydroxyaporphine, liensinine and asimilobine (Kulkarni and Juvekar, 2008). It has been reported to have anti-stress (Kulkarni and Juvekar, 2008), anti-obesity (Ono *et al*., 2006) and anti-oxidant (Rai *et al*., 2006), hepatoprotective (Sohn *et al*., 2003), anti-diabetic activity (Mukherjee *et al*., 1997a), anti-inflammatory (Mukherjee *et al*., 1997b), antipyretic (Mukherjee *et al*., 1996) activities.

The anti-malaria and antifungal activity of *N. nucifera* extract have also been reported (Agnihotri *et al*., 2008). The leaves have been indicated as part of the plant responsible for its anti-malarial activity (Li and Xu, 2008). In many Asian countries *N. nucifera* part was found in the recipe of food and folklore such as root and seed (La-ongsri *et al*., 2008). However, the *N. nucifera* pollen essential oil anti-microbial activity has never been reported. The present study deals with the antimicrobial activity of the essential oil extracted from *N. nucifera*.

MATERIALS AND METHODS

Sample collection identification and extraction: The *N. nucifera* was collected on May 2008 from Ayuthaya Province, Thailand. The plant was identified by Department of Biology, Faculty of Science, Mahasarakham University, Thailand. Ten gram of dried flowers were hydro-distilled in 500 mL water and repeat 3 times. The yield of vapor distillation was 0.5-1% of dried weight of dried plant's flowers. In this study used pooled of batches of the same condition of distillation throughout the studies.

Microbial cultures: Laboratory isolates of the pure culture of gram positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917) and gram negative bacteria (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumonia* ATCC 10031, *Pseudomonas aeruginosa* ATCC9721, *Proteus vulgaris* ATCC 13315) were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Antimicrobial sensitivity test

Agar disc diffusion test: The disc diffusion method used 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 (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 leave until the agar become rigid. The starter solution was swabbed on agar surface by using swab cotton.

N. nucifera essential oil was dissolved in olive oil (1.25, 2.5 and 5% v/v) 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 hour, the plates were incubated at 37 °C for 19 h. The NSS (normal saline solution) filled in the cylinder was used as control and 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 crude water extract of *N. nucifera* essential oil were determined by agar dilution method (Merck) (Lorian, 1996) while MBCs were determined by broth macro-dilution method were (Lorian, 1996). Gentamicin sulphate (Sigma Chemical Co., St. Louis, USA) was used as a reference antibiotic. 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 tube were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of ME 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 agar medium. Values were means of triplicate.

RESULTS AND DISCUSSION

The control growth of food borne pathogen bacteria such as *S. typhimurium*, *E. coli*, *S. aureus*, *Ps. aeruginosa* and *B. subtillis* are common in the tropical countries especially in country where poultry products are produced (Van Dijk *et al*., 2007). The present study has investigated anti-microbial activity of *N. nucifera* which used in food and folklore. Furthermore, *N. nucifera* has almost every part is edible which also mean less toxicity. Therefore incorporation of *N. nucifera* in to food as food born protection consider less toxic. In this study anti-microbial activity of *N. nucifera* pollen essential oil has been test against various bacteria include food born pathogen bacteria.

The anti-malarial and antifungal activity of *N. nucifera* extract has been reported (Agnihotri *et al*., 2008). In earlier study indicated that the leaves of *N. nucifera* may responsible for antibacterial activity (Li and Xu, 2008). However, the pollen of *N. nucifera* has never been investigated even it used as the ingredient of food and some folkloric medicine.

In this study the essential oil of *N. nucifera* were tested against various bacteria to screen the possibility to use it as food preservation additive. The *N. nucifera* pollen essential oil showed anti-microbial activity against gram negative *S. typhimurium* ATCC14028 and *E. coli* ATCC 25922 (Table 1). The MICs are 10 and 40 and MBCs are 20 and 80 ml L^{-1} (Table 2). In conclusion *N. nucifera* pollen essential oil show inhibitory effect on growth of food born pathogen bacteria in low concentration which indicated the possibility of used as food preservation additive.

Table 1: Diameter of zone of inhibition of bacterial organisms of *N. nucifera* pollen essential oil against various bacteria

		Inhibition zone diameter (mm)			
Bacteria	Gram	*N. nucifera* (5% v/v)	*N. nucifera* (2.5% v/v)	*N. nucifera* (1.25% v/v)	Gentamicin sulphate (10 μg mL^{-1})
S. aureus ATCC 25923	+	nz	nz	nz	21.0±1.00
S. epidermidis ATCC 12228	+	nz	nz	nz	19.8±1.73
M. luteus ATCC 9341	+	nz	nz	nz	19.0±0.61
B. subtillis ATCC 6633	+	nz	nz	nz	18.7±1.47
L. plantarum ATCC 14917	+	nz	nz	nz	22.3±1.85
E. coli ATCC 25922	-	19.3	nz	nz	20.6±0.88
K. pneumoniae ATCC 10031	-	nz	nz	nz	16.4±1.11
S. typhimurium ATCC 14028	-	17.5	12.5	nz	16.5±1.10
Ps. aeruginosa ATCC 9721	-	nz	nz	nz	17.9±1.61
P. vulgaris ATCC13315	-	nz	nz	nz	20.7±0.41

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

Table 2: The MICs and MBCs of *N. nucifera* pollen essential oil against various bacteria

	N. nucifera pollen essential oil		Gentamicin sulphate	
Bacteria	MIC (ml L^{-1})	MBC (ml L^{-1})	MIC (μg mL^{-1})	MBC (μg mL^{-1})
E. coli ATCC 25922	40	80	<0.5	nd
S. typhimurium ATCC 14028	10	20	<0.5	nd

nd: Not Determine

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