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Anti-Microbial Activities of *Millingtonia hortensis* Linn. Flowers Essential Oil

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Abstract: *Millingtonia hortensis* Linn. flowers have been extracted for essential oil using vapor distillation with 0.5-2% yield. The essential oil of *M. hortensis* Linn. was tested against various species of bacteria. The agar diffusion susceptibility test showed an inhibitory effect on 6 out of 10 tested strains. The growth of 4 of gram-positive bacteria (*S. aureus* ATCC 25923, *S. epidermidis* ATCC12228, *B. subtilis* ATCC6633 and *L. plantarum* ATCC14917) and 2 of gram negative bacteria (*E. coli* ATCC25922 and *P. vulgaris* ATCC13315) were inhibited by *M. hortensis* Linn. flower essential oil. The MICs (minimal inhibitory concentration) of *M. hortensis* Linn. flower essential oil are 0.5-2 and 1-4 ml L^{-1}, respectively. In this study *M. hortensis* Linn. flower essential oil showed broad spectrum for the anti-microbial activity at low concentration.

Key words: *Millingtonia hortensis* Linn., nosocomial infection, urinary tract infection, anti-microbial, essential oil

INTRODUCTION

Millingtonia hortensis Linn. is a tree which belong to family Bignoniaceae. In Thailand, *M. hortensis* Linn. dried flowers have been used for the cigarette ingredients to give sweet aroma and scent for relaxation. Moreover, it has been also used as Thai folklore for asthmas treatment. The hispidulin (6-methoxy-5,7,4'-trihydroxy-flavone) in this plant showed bronchodilator effect on rat's trachea (Anulakanapakorn *et al*., 1987). The extraction of *M. hortensis* Linn. leaf using polar solvent showed also a good antimicrobial activity (Jetty and Iyengar, 2000).

Furthermore, the extract of *M. hortensis* Linn. showed the RKO colon cancer cell line apoptosis inducing activity (Tansuwanwong *et al*., 2006, 2008) and antimutagenic activity of its flavonoid (Chulasiri, 1998) and hispidulin and hortensis (Chulasiri, 1992). Furthermore, the leaf extract of *M. hortensis* Linn. also showed lavacidal activity against three kinds of mosquito's lavae *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*, respectively (Kaushik and Saiini, 2008). Recently, *M. hortensis* Linn. essential oil has been used for the body massage and aroma therapy in Thailand. The study of its antimicrobial activity against pathogenic and normal flora has been investigated for the body massage and aroma therapy. In this study, antimicrobial activities of *M. hortensis* Linn. flowers essential oil against pathogenic and normal flora bacteria was evaluated.

MATERIALS AND METHODS

Plant Sample and Extraction

Millingtonia hortensis Linn. were collected and from Ayuthaya province, Thailand on May 2008. The plant was identified by Department of Biology, Faculty of Science, Mahasarakham University, Thailand. Ten gram of dried flowers were distilled in 500 mL water. The yield of vapor distillation are 0.5-2% of dried weight of the dried flowers. In this study, we used the batches of the same condition of distillation throughout the studies.

Tested Microorganism

Laboratory isolates of the pure culture of 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, *Pseudomonas aeruginosa* ATCC 9721) bacteria, were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Antimicrobial Assay

Agar Diffusion Susceptibility Test

Agar diffusion susceptibility determinations were 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 the surface of the agar slant with sterile 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 putted in cultivation plates and swabbed starter solution on agar surface by using swab cotton.

Millingtonia hortensis Linn. essential oil was dissolved in olive oil 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 saline solution (0.9% NaCl) 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 (Minimal Inhibitory Concentration) and MBCs (Minimal Bactericidal Concentration) Determination using Agar Dilution and Broth Macro Dilution Methods

MICs of *M. hortensis* Linn. essential oil was determined by agar dilution method (Merck) (Lorian, 1996) while MBCs were determined by broth macro-dilution method were (Lorian, 1996) and reference antibiotics gentamicin sulphate (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 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 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 agar medium. Values were means of triplicate.

RESULTS AND DISCUSSION

In earlier study has been reported that flavonoid (Hase, 1995) and alkaloid (Hase, 1966) from *M. hortensis* Linn. showed the bronchodilator effect. It was proven that hispidulin (6-methoxy-5, 7, 4'-trihydroxy-flavone) in this plant showed bronchodilator effect on rat's trachea (Anulakanapakorn *et al*., 1987). Moreover, the extract of *M. hortensis* Linn. showed the RKO colon cancer cell line apoptosis inducing activity (Tansuwanwong, *et al*., 2006) and antimutagenic activity of its flavonoid (Chulasiri, 1998) and hispidulin and hortensis (Chulasiri, 1992). The plant leaves water/ethanolic extract also showed inhibitory effect against *E. coli* and *S. typhimurium* (Jetty and Iyengar, 2000).

In this study, *M. hortensis* Linn. flower essential oil were tested against various bacteria. The agar diffusion susceptibility test of *M. hortensis* Linn. flower essential oil showed an inhibitory effect on 6 out of 10 tested strains. The growth of 4 of gram positive bacteria (*S. aureus* ATCC 25923,

Table 1: Inhibition zone diameters of *M. hortensis* Linn. flower essential oil against various bacteria

Bacteria	Gram	Inhibition zone diameter (mm)			Gentamicin sulphate (10 mg L^{-1})
		M. hortensis Linn. (4 mL L^{-1})	*M. hortensis* Linn. (2 mL L^{-1})	*M. hortensis* Linn. (1 mL L^{-1})	
S. aureus ATCC 25923	+	14.7±0.8	nz	nz	21.6±0.9
S. epidermidis ATCC 12228	+	13.9±0.7	nz	nz	20.9±0.9
M. luteus ATCC 9341	+	nz	nz	nz	20.7±1.0
B. subtilis ATCC 6633	+	15.6±0.3	nz	nz	20.2±1.8
L. plantarum ATCC 14917	+	12.5±0.7	nz	nz	19.3±0.9
E. coli ATCC 25922	-	23.5±1.6	17.3±1.6	14.6±0.9	21.2±1.0
K. pneumoniae ATCC 10031	-	nz	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	-	14.7±0.7	nz	nz	19.9±1.3

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

Table 2: The MICs and MBCs of *M. hortensis* Linn. flower essential oil against various bacteria

Bacteria	*M. hortensis* Linn.		Gentamicin sulphate	
	MIC (mL L^{-1})	MBC (mL L^{-1})	MIC (mL L^{-1})	MBC (mL L^{-1})
S. aureus ATCC 25923	1.0	2	<0.5	nd
S. epidermidis ATCC 12228	0.5	1	<0.5	nd
B. subtilis ATCC 6633	2.0	4	<0.5	nd
L. plantarum ATCC 14917	0.5	1	<0.5	nd
E. coli ATCC 25922	0.5	1	<0.5	nd
P. vulgaris ATCC 13315	2.0	4	<0.5	nd

nd = Not determine

S. epidermidis ATCC12228, *B. subtilis* ATCC6633 and *L. plantarum* ATCC14917) and 2 of gram negative bacteria (*E. coli* ATCC25922 and *P. vulgaris* ATCC13315) were inhibited by *M. hortensis* Linn. flower essential oil (Table 1). The MICs of *M. hortensis* Linn. flower essential oil are 0.5-2 and 1-4 ml L^{-1}, respectively (Table 2).

M. hortensis Linn. flower essential oil showed good inhibitory effect against various optimistic pathogen bacteria. The gram negative bacteria, *E. coli* and *P. vulgaris* causes urinary tract infection. The gram positive bacteria, *S. aureus* and *B. subtilis* causes food borne. This indicated that the *M. hortensis* Linn. showed broad spectrum of antibacterial activity at low concentration.

REFERENCES

Anulakanapakorn, K., N. Bunyapraphatsara and J. Satayavivad, 1978. Phytochemical and pharmacological studies of the flowers of *Millingtonia hortensis* Linn. F. J. Sci. Soc. Thailand, 13: 71-83.

Chulasiri, M., N. Bunyapraphatsara and P. Moongkandi, 1992. Mutagenicity and antimutagenicity of hispidulin and hortensin, the flavonoids from *Millingtonia hortensis* L. Environ. Mol. Mutagen., 20: 307-312.

Chulasiri, M., 1998. Mutagenicity and antimutagenicity of flavonoids extracted from *Millingtonia hortensis* L. J. Toxicol. Sci., 2: 224-228.

Hase, T., K. Ohtani, R. Kasai, K. Yamasaki and C. Picheansoonthorn, 1995. Revise structure of hortensin, a flavonoid from *Millingtonia hortensis*. Phytochemistry, 40: 287-290.

Hase, T., K. Ohtani, R. Kasai and C. Picheansoonthorn, 1996. Millingtonine an unusual glucosidal alkaloid from *Millingtonia hortensis*. Phytochemistry, 41: 317-321.

Jetty, A. and D.S. Iyengar, 2000. Antimicrobial activity of *Millingtonia hortensis* leaf extract. Pharm. Biol., 38: 157-160.

Kaushik, R. and P. Saini, 2008. Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. J. Vector Borne Dis., 45: 66-69.

Lorian, V., 1996. Antibiotics in Laboratory Medicine. 4th Edn., Williams and Wilkins, Baltimore, London, ISBN: 9780781749831.

Tansuwanwong, S., Y. Hiroyuki, I. Kohzoh and U. Vinitketkumnuen, 2006. Induction of apoptosis in RKO colon cancer cell line by an aqueous extract of *Millingtonia hortensis*. Asian Pac. Cancer Prev., 7: 641-644.

Tansuwanwong, S., H. Yamamoto, K. Imai and U. Vinitketkumnuen, 2008. Antiproliferation and apoptosis on RKO colon cancer by *Millingtonia hortensis*. Plant Foods Hum. Nutr. 10.1007/s11130-008-0094-8.