

Duangkamol Taemchuay 2010: A Study on *Centella asiatica* Linn. (Urban) Leaf Extracts Against *Staphylococcus aureus* in Bovine Mastitis. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Theera Rukkwamsuk, Ph.D. 121 pages.

The study aimed to identify asiatic acid in crude extracts of *C. asiatica* and to test the antibacterial activity of crude extracts against *S. aureus*.

Thin Layer Chromatography (TLC) method was developed to determine asiatic acid in *C. asiatica* using chloroform, methanol, ethyl acetate and water (30:5:5:1) as the mobile phase. The developed plate was sprayed with anisaldehyde-sulphuric acid reagent and the plate was determined asiatic acid with densitometer. The percentage of recovery was 99.70%, the detection limit was 0.76 ng and the quantitation limit was 2.60 ng.

Crude extracts of *C. asiatica* were tested for antibacterial activity against 30 isolates of *S. aureus* from milk samples of dairy cows. The antibacterial activity of crude extracts was tested by the disc diffusion test. The ethanol extracts and water extracts of *C. asiatica* had average inhibition zones ranged from 6.44-6.49 and 6.54-17.72 mm in diameter, respectively. The minimum inhibitory concentration (MIC) was determined by the modified resazurin microtiter-plate. The minimum bactericidal concentration (MBC) was determined by touching the loop from each well of MIC plate and streaking it on a mannitol salt agar. The ethanol extracts had an MIC₅₀ value of 8 mg/ml, the water extracts of leaf powder had an MIC₅₀ value of 32 mg/ml, and the water extracts of fresh leaves had an MIC value of 32-256 mg/ml. The ethanol extracts had an MBC value of 16 mg/ml. The water extracts could not kill *S. aureus*. Three DNA patterns of *S. aureus* were identified from 30 isolated from bovine mastitis by polymerase chain reaction (PCR) technique based on *coa* gene. The sequences of 30 isolates, representing 10 groups were determined. The 30 isolated were also tested for susceptibility to antimicrobial drugs using the agar disc diffusion test. The highest resistance was observed in β -lactam antibiotics, penicillin and ampicillin.

Student's signature

Thesis Advisor's signature