

# Antibacterial Activity of *Eupatorium ordortum* and *Centella asiatica* Crude Leaf Extracts Against *Staphylococcus aureus* ฤทธิ์ต้านแบคทีเรียของสารสกัดหยาบจากใบสาบเสือและใบบัวบกต่อ เชื้อสแตฟฟีโลคอคคัส ออเรียส

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## Abstract

Siam weed (*Eupatorium odoratum*) and Asiatic pennywort (*Centella Asiatica*) have been used in humans and animals to treat infectious diseases, especially on their skin infection and gastrointestinal intoxication. *Staphylococcus aureus* is a Gram-positive bacterium. It is caused by skin and soft tissue infections. Moreover, enterotoxigenic staphylococci are the major food-borne diseases which are produced by *S. aureus*. The research objective was to compare *E. odoratum* and *C. Asiatica* crude leaf extracts against *S. aureus* of Thailand Institute of Scientific and Technological Research (TISTR) 1466; (*S. aureus* TISTR 1466) using the paper disc diffusion method. Kanamycin 30 µg and sterile distilled water were positive and negative controlled, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured by the broth macrodilution method. MIC of extracted *E. odoratum* and *C. Asiatica* leaves were 6.25 mg/mL. Spread plate technique on mannitol salt egg yolk (MSEY) agar was estimated for minimal bactericidal concentration (MBC) while observed of the bacterium with no growth. MBC of extracted *E. odoratum* and *C. Asiatica* leaves were 25.0 and 12.5 mg/mL, respectively. Therefore, both plants could be a potential alternative for treating human and animal infectious diseases.

**Keywords:** Antibacterial Activity, *Eupatorium odoratum*, *Centella asiatica*, Crude Leaf Extracts and *Staphylococcus aureus*

## บทคัดย่อ

สาบเสือ (*Eupatorium odoratum*) และบัวบก (*Centella asiatica*) ถูกใช้รักษาโรคติดเชื้อในมนุษย์และสัตว์มาช้านาน โดยเฉพาะโรคติดเชื้อที่ผิวหนังและโรคในระบบทางเดินอาหารจากการได้รับสารพิษที่ *S. aureus* สร้างขึ้น สแตฟิโลคอคคัส ออเรียส (*Staphylococcus aureus*) เป็นแบคทีเรียแกรมบวก เป็นเชื้อสาเหตุในการติดเชื้อที่ผิวหนังและเนื้อเยื่อ นอกจากนี้ยังสามารถสร้างสารพิษซึ่งเป็นสาเหตุหลักในการก่อโรคอาหารเป็นพิษ วัตถุประสงค์ของการวิจัย เพื่อศึกษาเปรียบเทียบสารสกัดหยาบจากใบสาบเสือและใบบัวบกต่อการยับยั้งเชื้อ *S. aureus* TISTR 1466 โดยวิธีการแพร่ผ่านกระดาษ (paper disc diffusion) กานาไมซิน 30 ไมโครกรัม ถูกใช้เป็นตัวควบคุมเชิงบวก ส่วนน้ำกลั่นปราศจากเชื้อใช้เป็นตัวควบคุมเชิงลบ ค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งการเจริญของเชื้อ (MIC) สแตฟิโลคอคคัส ออเรียส ของสารสกัดหยาบจากใบสาบเสือและใบบัวบก มีค่าเท่ากับ 6.25 มิลลิกรัมต่อมิลลิลิตร การประเมินค่าความเข้มข้นต่ำสุดที่สามารถฆ่าเชื้อ (MBC) สแตฟิโลคอคคัส ออเรียส ใช้เทคนิคการเกลี่ยเพลท (spread plate technique) บนจานอาหารเลี้ยงเชื้อแข็ง MSEY โดยสังเกตจากเชื้อที่ไม่สามารถเจริญได้บนอาหารเลี้ยงเชื้อแข็ง ค่า MBC ของสารสกัดจากใบสาบเสือและใบบัวบกที่ได้คือ 25.0 และ 12.5 มิลลิกรัมต่อมิลลิลิตร ตามลำดับ ดังนั้น พืชทั้งสองชนิดนี้มีศักยภาพสามารถใช้เป็นทางเลือกในการรักษาโรคติดเชื้อในมนุษย์และสัตว์ได้

**คำสำคัญ:** ฤทธิ์ต้านแบคทีเรีย สาบเสือ บัวบก สารสกัดหยาบจากใบ สแตฟิโลคอคคัส ออเรียส



## Introduction

The leaves of Siam weed (*Eupatorium odoratum*) and Asiatic pennywort (*Centella asiatica*) have been used traditionally to cure abscess due to infectious diseases, leech bite and wound healing and widely used in Asia for treating a variety of diseases in human such as diarrhea, hepatitis, measles, toothache, syphilis, leucorrhoea and etc. (Hasnawati & Prawita, 2010; Zahara, Bibi & Tabassum, 2014; Prakash, Jaiwal & Srivastava, 2017). In Thailand, the extracted leaves of the plants also used in animals wound healing, against *S. aureus* in bovine mastitis and bio-pesticide in golden apple snail (Sintaweewarakul, Sintaweewarakul & Harnthavee, 2009; Taemchuay, Rukkwamsuk, Sakpuaram & Ruangwises, 2009; Nongnutch & Nanuam, 2015). Madecassic acid, asiatic acid, alpha-terpinene, alpha-copaene, beta-caryophyllene are some of the important bioactive compounds responsible for its antioxidant, antimicrobial,

antiulcer, antifilarial, antiviral and various other activities of *C. asiatica* (Zahara et. al., 2014). The essential oil obtained of the leaves and stem of *E. odoratum* are more than 19 types and the most dominated compound found are beta-cubebene, delta-cadinene and caryophyllene (Suwaibah, Zatilfarihiyah, Aslizah & Baharum, 2012). Therefore, phytochemicals both of extracted plants were used as bacterial inhibition especially *S. aureus* that contaminated on human and animal skin for reducing inflammatory and against foodborne pathogens (Hasnawati et al., 2010; Arumugam, Ayyanar, Pillai & Sekar, 2011; Dash et. al., 2011; Netala et. al., 2015; Wong & Ramli, 2021). In this study, extracted of *E. odoratum* and *C. asiatica* for inhibition of *S. aureus* in vitro were a compared.

## Objective

Research objective was to compare *E. odoratum* and *C. asiatica* crude leaf extracts against *S. aureus*.

## Materials and Methods

### Plants Collection and Preparation

*E. odoratum* and *C. asiatica* leaves were collected from Nong Katum village, Muen Wai sub-district, Mueang Nakhon Ratchasima district, Nakhon Ratchasima province in November, 2020. The samples were washed, chopped, dried at 60 °C in hot air oven and blended to fine powder and kept in sterile plastic zip lock bag at room temperature until use.

### Plants extraction

Two-hundred grams of each powder was soaked in 1,000 mL of 95% ethanol for a week, then filtrated through paper filter with Whatman No.1 paper. The evaporation of the solvent was poured the supernatant into round-bottom flask then put in evaporator (DLAB, RE100-PRO, China) under-temperature at 40-50 °C and pressure at 200-220 millibar until dry. Crude extracts were collected, weighed and kept at 4 °C until use. The crude extracts were examined for antibacterial activity.

### Percentage yield

The yield of extraction was calculated by following equation: %Yield = Weight of dry extract/Weight of dry plant material × 100%

## Bacterial Culturing

*S. aureus* of Thailand Institute of Scientific and Technological Research (TISTR) 1466; (*S. aureus* TISTR1466) was used as reference strain. Trypticase soy broth (TSB, Difco, U.S.A.) supplemented with 10% NaCl was enriched for the bacterium cells and incubated at 37 °C for overnight. The inoculum was streaked on saline trypticase soy agar (TSA, Difco, U.S.A. plus 10% NaCl) and incubated at 37 °C for 24 h. The single colony was resuspension in TSB+10% NaCl broth and incubated at 37 °C for 16-18 h for antimicrobial test.

## Antibacterial Test

The suspension of *S. aureus* TISTR 1466 strain in TSB+10% saline after incubated at 37 °C for 16-18 h was adjusted to 10<sup>8</sup> colonies forming unit--cfu when compared to 0.5 McFarland standard solution then swabbed onto the surface of mannitol salt egg yolk agar (MSEY; Difco, U.S.A.) using a sterile cotton swab for paper disk diffusion test. Kanamycin 30 µg of concentration and sterile distilled water was used as positive and negative control, respectively. *E. odoratum* and *C. asiatica* crude leaf extracts were dropped on the sterile paper disk. The paper disks were made from sterile filter papers (Whitman No.1) which were the same size as the antibiotic disk diameter. MSEY duplication plates were incubated at 37 °C for 24 h. The inhibition zones diameter was measured in millimeters. The Minimum Inhibitory Concentration--MIC and Minimum Bactericidal Concentration--MBC were measured by broth macrodilution method. The tubes of TSB were used for two-fold dilution of each extract substance. The inoculum was added into the dilution tubes with a proportion of 1:1 then incubated at 37 °C

for 24 h. The bacterial growth was observed with broth turbidity after incubation. The lowest concentration tube of the extracted and no growth of the bacterium (clear broth) was MIC according to Phaiboon, Pulbutr, Sungthong & Rattanakit (2019). MBC was detected using spread plate technique on MSEY of each tube and incubated at 37 °C for 24 h. MBC was indicated of bacterium with no growth on agar plate.

## Results

Yield percentage of *E. odoratum* and *C. asiatica* leaves powder of *E. odoratum* and *C. asiatica* were extracted using 95% ethyl alcohol as a solvent. The ratio of powder per solvent was 1:5 (w/v) and leave them at room temperature for

a week. Solvent evaporation using evaporator (DLAB, RE100-PRO, China), the yield percentage of *E. odoratum* and *C. asiatica* was 2.32 and 2.90, respectively. Picture of *E. odoratum* and *C. asiatica* were used in this study show in Figure 1.

*E. odoratum* and *C. asiatica* crude leaf extracts were tested for inhibition of *S. aureus* TISTR 1466. The extracted concentrations of 100, 10, 1, 0.1 and 0.01, mg/mL were loaded on paper disks then put on MSEY which were swabbed with the bacterium. The results, showed that all concentrations of both crudes extracted inhibited *S. aureus* TISTR 1466. The inhibition zone diameter, MIC and MBC of *E. odoratum* and *C. asiatica* crude leaf extracts against the bacterium strain ranged from 0.8 to 23.0 mm as shown in Table 1.



**Figure 1.** Fresh leaves of *E. odoratum* and *C. asiatica* were used in this study

**Table 1.**

*The inhibition zone diameter, MIC and MBC of E. odoratum and C. asiatica crude leaf extracts against S. aureus TISTR 1466*

| Crude leaf extracts concentration          | Inhibition zone diameter (mm) |                    |
|--------------------------------------------|-------------------------------|--------------------|
|                                            | <i>E. odoratum</i>            | <i>C. asiatica</i> |
| 100 mg/mL                                  | 19.00                         | 23.00              |
| 10 mg/mL                                   | 17.00                         | 18.00              |
| 1 mg/mL                                    | 15.00                         | 16.00              |
| 0.1 mg/mL                                  | 10.00                         | 12.00              |
| 0.01 mg/mL                                 | 0.80                          | 10.00              |
| Positive control (Kanamycin 30 µg/mL)      | 36.00                         | 36.00              |
| Negative control (Sterile distilled water) | 0.00                          | 0.00               |
| MIC                                        | 6.25 mg/mL                    | 6.25 mg/mL         |
| MBC                                        | 25.00 mg/mL                   | 12.50 mg/mL        |

Crude extract concentration 100 mg/mL of *C. asiatica* was the strongest to inhibit *S. aureus* TISTR 1466 with inhibition zone 23 mm

#### Minimal Inhibitory Concentration--MIC of *E. odoratum* and *C. asiatica* crude leaf extracts using multiple tubes technique

Multiple tubes of two-folded dilution were used for MIC test of both *E. odoratum* and *C. asiatica* crude leaf extracts against *S. aureus* TISTR 1466. The extracted concentrations were ranged with 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/mL. One milliliter of *S. aureus* TISTR 1466 was added into all tubes then observed after incubated at 37 oC for 24 h. MIC of *E. odoratum* and *C. asiatica* were 6.25 mg/mL with clear broth.

#### Minimal Bactericidal Concentration--MBC of *E. odoratum* and *C. asiatica* crude leaf extracts using spread plate technique

According to MIC of *E. odoratum* and *C. asiatica* crude leaf extracts against *S. aureus* TISTR 1466 was 6.25 mg/mL. Therefore, the lowest concentration of each extracted was observed between 6.25 and 50 mg/mL for MBC testing. Spread plate technique followed as: *E. odoratum* and *C. asiatica* crude leaf extracts concentration of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/mL were mixed with *S. aureus* TISTR 1466 then spreaded on MSEY agar using sterile spreader. After incubation, MBC of *E. odoratum* and *C. asiatica* extracted were 25 and 12.5 mg/mL, respectively.

## Discussion

From the results of *E. odoratum* and *C. asiatica* crude leaf extracts for inhibition of *S. aureus* related to previous studies. Both extracts were the same MIC but MBC of *C. asiatica* was lower than *E. odoratum* about two-folds. *C. asiatica* could be inhibit the bacterium better than *E. odoratum*. However, the research indicated and proved that both plants were inhibited to other bacteria and fungi also, such as *Bacillus cereus*, *Escherichia coli* O157: H7, *Salmonella enterica* serovar Typhimurium, *S. aureus*, *Aspergillus niger* and *Candida albicans* (Wong et al., 2021). These extracted plants can be alternative used in both human and animal or even a pesticide (Sintaweewarakul et. al., 2009; Taemchuay et. al., 2009; Nongnutch et al., 2015). Many types of bioactive compounds form *E. odoratum* and *C. asiatica* were studied (Zahara et. al., 2014; Suwaibah et. al., 2012). Therefore, both plants could be potential alternative for promoting and treating for human and animal infectious disease.

## Conclusion

*E. odoratum* and *C. asiatica* leaves were collected from Nakhon Ratchasima province in November, 2020. The samples were fined and extracted using 95% ethyl alcohol for a week then evaporated in evaporator for collected of crude extracted. *E. odoratum* and *C. asiatica* yield percentage were 2.32 and 2.90, respectively. Paper disc diffusion method was used to determine the inhibition. The inhibition zone diameter of plant extracts against the bacterium strain ranged from 0.8 to 23.0 mm. Crude extract concentration 100 mg/mL of *C. asiatica* was the strongest to inhibit *S. aureus* TISTR 1466. Both MIC of *E. odoratum* and *C. asiatica* was 6.25 mg/mL whereas BMC were 25 and 12.5 mg/mL, respectively. Both plants could be used as Thai medicinal for *S. aureus* infection.

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