

Cell leakage mechanism and time-kill studies on *Staphylococcus aureus* after exposure to ethanol leaf extract of *Muntingia calabura* L

ABSTRACT

Purpose: To determine the effect of the ethanol leaf extract of *M. calabura* (EEMC) on cell leakage and time-kill against *S. aureus*. Methods: The leaves were macerated with 96 % ethanol (1:8; w/v) for 27 h to produce EEMC. Chemical compounds of EEMC were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Various concentrations of EEMC (12.5; 25; 50; 100 mg/mL) were tested to determine the Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. aureus*. Furthermore, EEMC was tested for its effect on cell leakage, changes in extracellular electrical conductivity, and time-kill against *S. aureus*. UV-spectrophotometer was used to test for leakages of nucleic acid, protein, DNA, and RNA, while atomic absorption spectrophotometer was used to evaluate leakage of potassium ion (K^+). Results: The MIC and MBC of EEMC against *S. aureus* were 10 % w/v (100 mg/mL). The highest cell leakage occurred in *S. aureus* exposed to 2x MIC, with leakages of protein, DNA, RNA, and K^+ reaching 137.79 ± 58.99 , 2298 ± 263.26 , 1839 ± 210.61 and 770.86 ± 40.11 $\mu\text{g/mL}$ respectively. The EEMC (1x MBC and 1.5x MBC) killed *S. aureus* in 24 h. Analysis of LC-MS/MS of EEMC showed that flavonoids (48.33 %) followed by anthraquinones (16.10 %) were the major classes of compounds present in the extract. Conclusion: The ethanol leaf extract of *M. calabura* kills *S. aureus* by inducing cell leakage possibly due to flavonoids and anthraquinones contained in it. The extract should be further isolated and its active principles with potent antibacterial properties developed for therapeutic applications.