

**Title: Assessment of antimicrobial activity of carvacrol and thymol against *Staphylococcus aureus***

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**Resume**

*Staphylococcus aureus* is one of the major bacterial pathogens related to food-poisoning that results from the consumption of foods containing sufficient amounts of preformed enterotoxin. Microbial resistance to antibiotics associated to negative perspective of consumers to synthetic substances leads to development of new strategies to control bacterial growth, such as the use natural compounds. This study evaluated the antimicrobial activity of carvacrol and thymol, compounds of thyme and oregano essential oils, against *Staphylococcus aureus* ATCC 14028. Minimum Inhibitory Concentration (MIC) was performed inoculating 10 µL of bacterial culture (final inoculum 5.10<sup>5</sup> CFU/mL) in 96-well microtiter plates containing carvacrol or thymol at 19 to 5000 µg/mL. Plates were incubated at 35 °C for 24 h and MIC was defined as the lowest concentration of compounds required to inhibit bacterial growth. Minimal Bactericidal Concentration (MBC) was performed inoculating 10 µL from wells without bacterial growth on Baird Parker Agar. Plates were incubated at 35 °C for 48 h and MBC was defined as the lowest concentrations of compounds where no bacterial growth was visualized on agar plates. Time-kill curve method was performed inoculating 100 µL of bacterial culture (final inoculum: 6.10<sup>5</sup> CFU/mL) to tubes containing 10 mL of MHB supplemented with carvacrol at 0.75 x MIC, MIC and 2 x MIC. MHB tubes non-supplemented with carvacrol were used as control. Tubes were incubated at 35 °C for 48 h. At interval times of 0, 3, 6, 12, 24, 48 h, 100 µL of each tube were collected, diluted in saline solution, plated on Mueller Hinton Agar and incubated at 35 °C for 24 h. Carvacrol showed CIM and CBM values of 312 µg/mL. Thymol was not able to inhibit bacterial growth at the maximum concentration tested, thus time-kill curve assay was not performed to this compound. After 48 h of incubation in time-kill curve assay, *S. aureus* population reached up 8.0 log<sub>10</sub> CFU/mL. Carvacrol at 0.75 x MIC maintained bacterial population compared to initial inoculum and MIC concentration decrease initial inoculums from 5 to 3.7 log<sub>10</sub> CFU/mL. No viable cells were recovered after 6 h of treatment with carvacrol at 2 x MIC. This study showed the antimicrobial properties of carvacrol, suggesting it could be used as alternative to *S. aureus* control.

**Keywords:** *Staphylococcus aureus*, carvacrol, thymol, time-kill curve assay.

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