

Thyme (*Thymus vulgaris L.*) Essential Oil (TEO) as a Tool for Mastitis Control in Small Ruminants: In vitro evaluation of antimicrobial activity and inhibition activity of biofilm production

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Abstract

Staphylococcus aureus and coagulase-negative staphylococci (CNS) are some of the main causes of mastitis in sheep. The ability of *Staphylococcus* spp. to form biofilms *in vivo* is considered an important virulence factor that could explain cases of mastitis refractory to antibiotic treatments.

The aim of the research was to evaluate the antibacterial and anti-biofilm-forming activity of Thyme Essential Oil (TEO) at concentrations of 9.28mg/mL, 4.64mg/mL, and 2.32mg/mL (w/v), against clinical strains of staphylococci isolated from ovine mastitis.

Two reference strains (ATCC25923 and ATCC11622) and 12 clinical isolates (6 *S. aureus* and 6 CNS) have been studied to assess the antimicrobial and anti-biofilm activity of TEO using the microdilution broth method.

Biochemical and molecular methods were used to identify the isolated bacteria and the disk diffusion method was employed to determine their antimicrobial resistance profile. The antibacterial efficacy was evaluated by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), while the biofilm inhibition was assessed by minimum biofilm inhibitory concentration (MBIC) by measuring the absorbance of Crystal Violet at 570nm, as previously described (1). Our results showed bactericidal efficacy of TEO with MIC and MBC values of 4.64mg/mL, while a total inhibition of biofilm production was found at sublethal concentrations of 2.32mg/mL.

These preliminary results demonstrated the antimicrobial and anti-biofilm efficacy of TEO *in vitro* against tested bacterial strains. Further studies are needed to confirm the *in vivo* reproducibility of these data in order to consider TEO as a valid alternative to classical antibiotic therapies and, consequently, to counteract the onset of antimicrobial resistance.

Keywords

Mastitis; Biofilm; Thyme Essential Oil; *Staphylococcus* spp.

Reference

1. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods*. 2000;40(2):175–179. doi: 10.1016/S0167-7012(00)00122-6.