

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

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Introduction

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 activities 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.

Materials and Methods

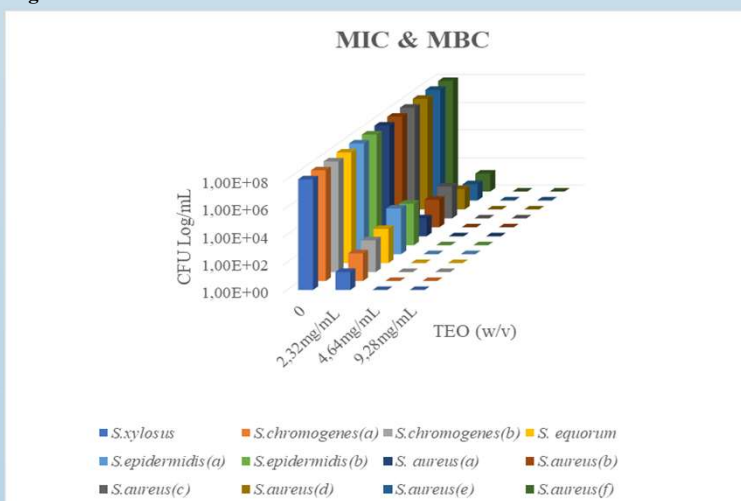
The antimicrobial and anti-biofilm activities of TEO were evaluated against 12 clinical isolates (6 *S. aureus* and 6 CNS) and two reference stains (ATCC25923 and ATCC11622) using the microdilution broth method.

The isolated bacteria were identified using biochemical and molecular methods while the antimicrobial resistance profile was determined setting the disk diffusion method. 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).

Results

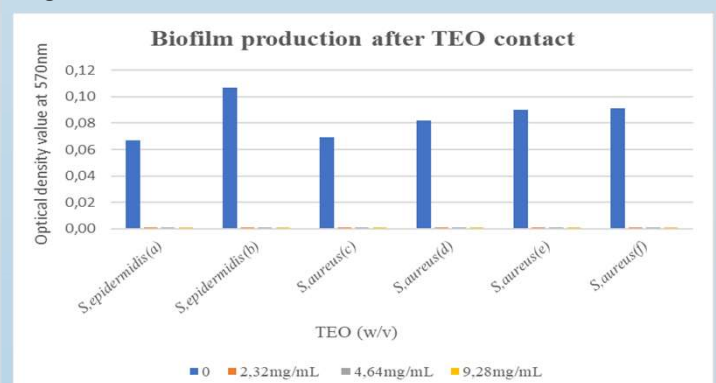
A complete inhibition of bacterial growth was observed for MIC values of 4.64 mg/mL (w/v). These values were confirmed in MBC (Fig.1)

Fig.1 MIC and MBC results



A total inhibition of biofilm production was found at sublethal concentrations of 2.32mg/mL (fig.2)

Fig.2 Biofilm results



Conclusions

These preliminary results demonstrated that TEO can be used to test *in vitro* the antimicrobial and anti-biofilm activities against the bacteria. 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 avoid the antimicrobial resistance development.

References

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.