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Session: The natural and bio-inspired drug universe

Title: Antibacterial activity of marine-derived chitosan and plant-derived cajeput oil as loaded blended films in *Staphylococcus aureus* and *Pseudomonas aeruginosa*-enriched settings

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Abstract

Chronic wounds (CW) have numerous entry ways for pathogen invasion and prosperity, damaging host tissue and hindering tissue remodelling. Essential oils (EOs) exert quick and efficient antimicrobial (AM) action, unlikely to induce bacterial resistance. Cajeput oil (CJO) has strong AM properties, namely against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as previously established by the team (DOI: 10.3390/antibiotics9060314). Chitosan (CS) is a natural and biodegradable cationic polysaccharide, widely known for its AM features. CS (100-300 kDa; DA of 9.6±1.4%) and PVA (72 kDa, 88% hydrolyzed) films (ratio 30/70; 9%wt) were prepared by solvent casting and phase inversion method (similarly as in DOI: 10.1002/app.48626). CJO was added to CS solution before blending it with PVA, with loading amount of 1 and 10 wt% in relation to total polymeric mass. Loaded films with 0.89 ± 0.05 and 1.14 ± 0.10 mm in thickness were obtained, respectively, 23 and 57% thicker than the unloaded films. Degree of swelling (%) and porosity also increased. Films chemical composition and thermal stability reinforce the achievement of loaded blended films. AM activity was evaluated through the agar diffusion assay and time kill kinetics, with the biomaterials incubated with *S. aureus* and *P. aeruginosa*. Thin inhibitory zones were observed with films placed in direct contact each of the bacterium. CS films alone showed an outstanding AM activity against both bacteria, with 1h being sufficient to eradicate all *P. aeruginosa* colony traces. Still, CS/PVA blended films carrying 1/10% CJO, having improved mechanical properties than CS films alone, resulted in 2/3 (*S. aureus*) or 3/4 (*P. aeruginosa*) log reduction in 24h of contact. This study is a first proof of concept that CJO can be dispersed into CS/PVA films and show bactericidal effects against both bacteria species, notably when combined with CS, this way opening new avenues for CW therapeutics.

Keywords: Bactericidal; marine-derived polymers; natural bioactive agents; drug delivery systems; blended films.