Antimicrobial Activity of Selected Natural Antimicrobials for Potential Use in the Development of Antimicrobial Active Packaging

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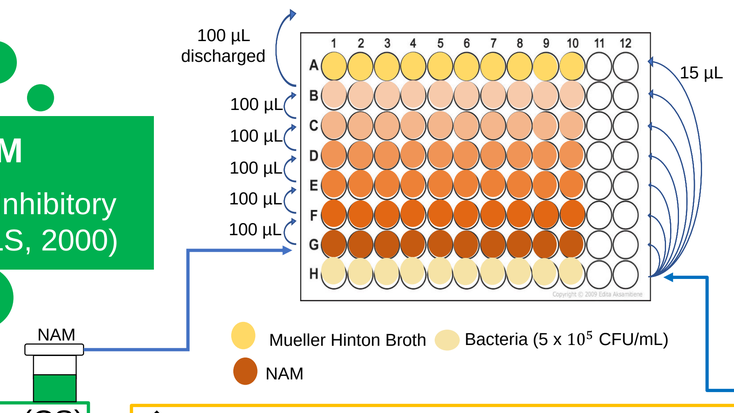
Introduction

Natural antimicrobials (NAM) have been widely used as polymer additive for food contact applications and could potentially be used for the development of antimicrobial active packaging (AAP) materials. However, higher concentrations of individual NAM are needed to inactivate bacteria, and this can affect the organoleptic characteristics of the food product. Enhanced antimicrobial activity (AA) can be achieved through the combination approach of different NAM and used as active agents in food packaging applications (Pandey *et al.*, 2021). The application of the enhanced combined antimicrobials for the development of AAP materials offers huge potential to enhance the antimicrobial properties of food packaging materials due to their ability to control microbial spoilage and increase the shelf-life of the packaged food product therefore reducing food waste. The objective of this study was to determine the antimicrobial activity of selected NAM and apply the combination approach to develop NAM with enhanced AA for Antimicrobial Packaging Applications.

Material & Methods

Screening of NAM

Determination of Minimum Inhibitory Concentration (MIC) (NCCLS, 2000)



- Low molecular weight chitosan (CS)
- Thyme essential oil (TEO)
- Commercially available mix of organic acids Articoat-DLP-02® (ART) & Sulac-01® (SUL)

- *Staphylococcus aureus* (*S. aureus*):(NCDO 949)
- *Escherichia coli (E. coli)*:(NCIMB 11943)
- *Pseudomonas fluorescens* (*P. fluorescens*):(NCIMB 9046)
- Salmon microflora

Checkerboard test

Determine best combination of MIC and Fractional Inhibition Concentration (FIC) index (Leber, 2016)

$$FIC\ \text{ART} = \frac{MIC\ (\text{ART+TEO}\ combined)}{MIC\ (\text{ART}\ alone)}$$

$$\text{FIC TEO} = \frac{\text{MIC (TEO+ART combined)}}{\text{MIC (TEO}\ alone)}$$

Time-kill test

- Confirm synergistic effect
- Bacteriostatic or bactericidal effect (CLSI Methods M26-A, 1999)

Combined NAM with enhanced AA

Synergy: FICI ≤ 0.5
Antagonism :FICI > 4
Additivity :FICI > 0.5-4

Results and Discussion

- The best NAM with highest AA against all assessed pure bacteria strains and fish microflora were CS, TEO and SUL with MIC of 0.125 mg/ml, 0.313 mg/mL and 3.75 mg/mL respectively. For ART, MIC ranged from 25 mg/mL (S. *aureus)* to 50 mg/mL (*P. fluorescens, E. coli* and salmon microflora)
- Compared to pure bacteria cultures, salmon microflora was more resistant to TEO (0.5 mg/mL) (**Table 1**).
- A synergistic effect (FIC Index ≤0.5) was noticed when ART was combined with TEO, decreasing significantly TEO concentration by 50-74% than when they are used alone. This is important in reducing impact of essential oil on organoleptic properties.
- A synergistic effect was similarly found in CS+ART combination against pure bacteria cultures and salmon microflora (**Table 2)** with exception of *S. aureus* (FIC index 0.63) (Data not shown)**.** Additivity effect (FIC Index 0.75) was noticed when CS+TEO was combined.
- The time-kill assay of the best combinations obtained using the checkerboard assay confirmed the synergistic effect for the combinations with FIC Index ≤0.5 (CS+ART) (**Figure 1**) and (TEO+ART) (**Figure 2**) with a (2 log reduction) compared to control, respectively. Furthermore, the results confirmed that combinations with synergistic effect have a bactericidal effect during the 24-h time-kill test (**Figures 1&2**)

Table 1. MIC of individual NAM against selected pure culture bacterial strains and salmon microflora*

Microbial species	MIC (mg/mL)			
	CS	TEO	SUL	ART
S.aureus	0.125	0.313	3.75	25
E. coli	0.125	0.313	3.75	50
P.fluorescens	0.125	0.313	3.75	50
Salmon microflora	0.125	0.5	3.75	50

*All values are means of analysis of triplicates samples

Table 2. FIC of combined NAM against selected pure bacteria strains and salmon microflora using checkerboard assay*

Bacteria	MIC (mg/mL) Best Combination		FIC Index FIC		≤ 0.5	Combination effect
	CS	ART	CS	ART		
E. coli	0.060	12.50	0.250	0.300	0.50	Synergy
P. fluorescens	0.030	12.50	0.250	0.250	0.50	Synergy
Salmon microflora	0.016	12.50	0.400	0.100	0.50	Synergy
	TEO	ART	TEO	ART		
S. aureus	0.080	6.250	0.250	0.250	0.50	synergy
E. coli	0.080	12.50	0.250	0.250	0.50	synergy
P. fluorescens	0.080	12.50	0.250	0.250	0.50	synergy
Salmon microflora	0.160	12.50	0.300	0.200	0.50	synergy

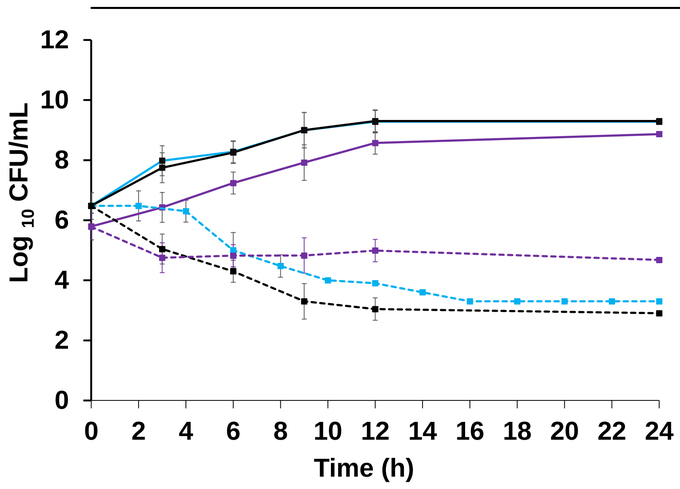


Figure 1. Time-kill curves of the combination CS+ART against *E. coli* (····), *P. fluorescens* (···) or salmon microflora (···) as compared to control *E. coli* (—),P. *fluorescens* (—),and salmon microflora (—) respectively.

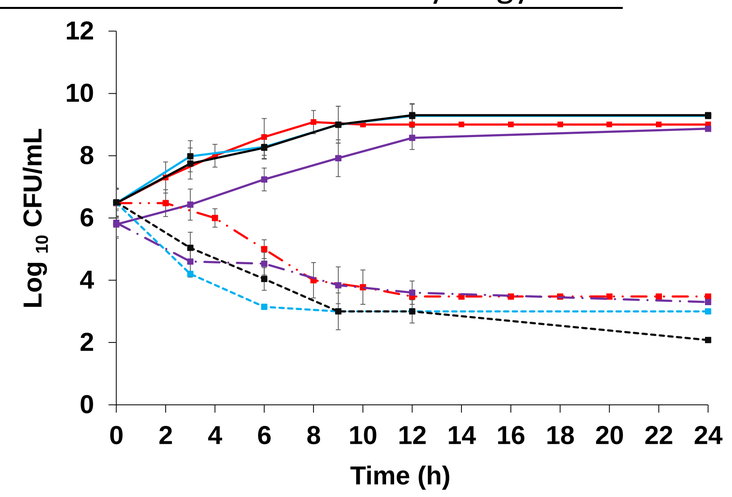


Figure 2. Time-kill curves of the combination TEO+ART against *S. aureus* (····), *E. coli* (····), *P. fluorescens* (····) or salmon microflora (···) as compared to control *S. aureus* (—), *E. coli* (—), *P. fluorescens* (—) and salmon microflora (—) respectively.

Conclusions

- NAM with high AA were CS, TEO and SUL
- A significant reduction in antimicrobial concentration was achieved using combined approach i.e., in ART+CS and ART+TEO combinations, MIC reduced
- The combined NAM had a wide antimicrobial spectrum against Gram-positive and Gram-negative bacteria and salmon microflora
- The results found in this study provide potential for using the developed antimicrobials with enhanced AA for the development of antimicrobial active packaging systems to extend the shelf-life of food products and hence improve sustainability by reducing food waste.

Future work

Integration of the developed natural antimicrobials with enhanced AA, using the combined approach, into commercially used packaging materials to develop advanced multi-functional packaging materials with active features able to extend the shelf-life of food products and reduce food waste.

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