Assessment of essential oil-loaded nanofibrous mats against the *Escherichia* virus MS2, a mimic of SARS-CoV-2, for potential applications as inner layers in individual protection masks

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Introduction

In December 2019, a novel strain of coronavirus, SARS-CoV-2, was identified. Infected patients revealed symptoms of fever, cough (dry), sore throat, and fatigue, which began manifesting after 5 days of incubation. Hoping to prevent transmission, many countries adopted a mandatory mask use in closed public spaces. However, most mask options display a passive action against COVID-19. To overcome such restrictions, this work proposes the incorporation of anti-viral essential oils (EOs) loaded onto a nanofibrous layer that can be adapted to both community and commercial masks.

EOs Drawbacks
- cytotoxic at increased concentrations, which prevents systemic delivery;
- present low resistance to degradation by external factors (e.g., temperature, light, moisture);
- highly volatile in their free, unloaded form.

Materials and Methods

Electrospinning

Spinning technique that allows the production of continuous, homogeneous nanofibers.

Polymeric solution preparation

| Polyacrylactone (PCL) | Cellulose Acetate (CA) |

PCL 14%wt in chloroform/dimethyl formamide (9/1 v/v)
PCL/CA 14%/10%wt (3/1 ratio) in acetone/dimethyl formamide (2/1 v/v)

Processing conditions

PCL = 12.0 kV, speed of 0.7 mL/h, and 17 cm of distance to collector. PCL/CA = 24.7 kV, speed of 3.2 mL/h, and 21 cm of distance to collector

EOs Minimum Inhibitory Concentrations (MICs)

20 EOs were tested – selected based on their antimicrobial action:
- Amyris; Cajeput; Cinnamon leaf; Citronella; Clove; Eucalyptus; Frankincense; Geranium; Himalayan cedar; Lavandin; Lemongrass; Niaouli; Orchid; Palmarosa; Patchouli; Rosemary; Sage; Star anise; Tea tree oil; Wintergreen.

Results and Discussion

Minimum inhibitory Concentrations (MIC)

List of most effective oils tested against MS2 virus at 1x10^7 PFUs/mL:

<table>
<thead>
<tr>
<th>EOs</th>
<th>MS2 virus (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td>Lemongrass</td>
<td>356.0</td>
</tr>
<tr>
<td>Niaouli</td>
<td>365.2</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>586.0</td>
</tr>
</tbody>
</table>

Nanofiber Morphology and Diameters

Degree of Swelling (DS) and Hydrophobicity

DS determined in simulated body fluid:
- DS of PCL = 74.84%
- DS of PCL/CA = 55.30%

Hydrophobicity in distilled water

EOs Incorporation and Antimicrobial Testing (ongoing)

Mats were loaded with the EOs by immersing the mats in a MIC concentrated EO/ethanol solution for 72 h (saturation). Only 10% of the oil was bonded to the fibers.

Antimicrobial testing via halo determination, verified their diffusion abilities. More importantly, time-kill kinetics testing of the loaded mats attested to the EOs capability to fight the virus MS2 even when bonded to the nanofibers – data based on one repetition (require confirmation).

Conclusions: Data demonstrated the potential of these EOs-loaded PCL/CA nanofibers mats to work as COVID-19 active barriers for individual protection masks.