INTRODUCTION

• Clostridium difficile causes infections of the gastrointestinal tract.1
• Disease severity can range from self-limiting diarrhea to severe manifestations such as pseudomembranous colitis and toxin megacolon.2
• C. difficile infection (CDI) has been managed with the conventional antimicrobials metronidazole (for mild or moderate CDI) and vancomycin (for severe CDI).3
• Several issues are associated with the use of conventional agents, including a high recurrence rate of 20–25% and reduced in vitro efficacy.1
• Natural compounds generally have broad-spectrum antimicrobial activity with several showing activity against C. difficile.4
• Compounds derived from plant extracts have great antimicrobial potential against drug-resistant microorganisms and, unlike conventional antimicrobials, they are less susceptible to the development of antimicrobial resistance.4,5

OBJECTIVE

This study aimed to investigate the mechanism of action of natural occurring compounds and plant extracts against C. difficile.

METHODS

The mechanism of action of five compounds with bactericidal activity (cinnamon root powder, peppermint oil, trans-cinnamaldehyde, menthol, and zingerone) and four with bacteriostatic activity (fresh garlic bulb extract, garlic clove powder, Leptospermum honey and allincin) against two C. difficile strains were investigated.

ASSAYS:
3. Inhibitory effect of compounds on prokaryotic translation/ protein synthesis. An in vitro transcription/translation experiment using Promega’s E. coli system was further carried out to represent DNA linked to the Steady-Glo®.
4. Detection of antimicrobial cross-resistance: broth microdilution, comparing the MICs of products against a panel of antimicrobial C. difficile strains previously characterised for AMR phenotype and associated genotype.

RESULTS

Time-kill kinetics

Table 1. Mean log10 cfu/ml reductions for C. difficile NCTC 13366 following exposure to treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Log reduction (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Peppermint</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Zingerone</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

ATP-leakage assay

Figure 2. Time-kill assay

Figure 3. Prokaryotic translation/protein synthesis

Cell permeability assays

Table 2. Effect of products on C. difficile NCTC 13366, measured by protein leakage and propidium iodide uptake assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein leakage assay (μg/mL)</th>
<th>Propidium iodide uptake assay (fluorescence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
</tr>
<tr>
<td>Control</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>1.2 ± 0.1</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Peppermint</td>
<td>1.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Menthol</td>
<td>0.8 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Zingerone</td>
<td>0.6 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
</tbody>
</table>

Detection of antimicrobial cross-resistance

Table 3. MIC values for products against antimicrobial resistant and susceptible C. difficile

Detection of antimicrobial cross-resistance

Overall, this study provides a fundamental framework regarding the possible mechanism of action of natural occurring antimicrobials against C. difficile. The findings indicate that damage to the cytoplasmic membrane may contribute to the mechanism of action of several naturally occurring antimicrobials against C. difficile. Also, a lack of cross-over mechanisms of resistance between standard antibiotics and natural compounds are shown. Further studies are required to determine the efficacy of these compounds in vivo.

REFERENCES


CONCLUSIONS

The time-kill assay showed a >3 log10 reduction in C. difficile counts by all five bactericidal compounds after 24 h. Peppermint oil at ≥ 1× MIC resulted in a logreduction of ≥3 against both log- and stationary-phase C. difficile after 24 h. A similar pattern of killing with a reduction in bacterial counts was observed with trans-cinnamaldehyde at almost all concentrations.

The ATP-leakage assay showed that all five bactericidal compounds at most concentrations significantly reduced the intracellular ATP after 1 h of incubation (P ≤ 0.001). The extracellular ATP was increased significantly by all five antimicrobials at all concentrations after 2 h (P < 0.001).

Inhibition of protein synthesis

Streptomycin sulfate and tetracycline were used as positive controls and both inhibited protein synthesis/prokaryotic translation. Other than streptomycin and tetracycline, Leptospermum honey (MGO 514+) was the only treatment that showed a reduction in RFU ratio compared to untreated controls (SDW and DMSO) (P ≤ 0.04). Treatment with all natural compounds at most tested concentrations resulted in a significant increase in propidium iodide fluorescence after 6 h of exposure (P < 0.05).

None of the compounds showed elevated MICs against antibiotic-resistant strains of C. difficile harbouring DNA gyrase mutations, or 5-fluorouracil transposons carrying ermB and tetM, suggesting that antibiotic resistance mechanisms are not cross-protective for natural products.