The antimicrobial activity of various essential oils against Gram-negative and Gram-positive bacteria.

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INTRODUCTION

In a time when antibiotics are ceasing to work as effectively as they once did, the need for alternative antimicrobials is increasing. Essential oils (EOs) have long been known to possess antimicrobial properties, and it is within this group of natural plant-derived volatiles that a novel approach to combatting pathogenic bacteria may be found. Despite EOs known antimicrobial properties, they are still considered novel and thus research is required to fully grasp their potential and their application within clinical settings. This study has been useful in demonstrating the activity of EOs against several bacterial species, many of which are clinically relevant and have known drug resistance, thus highlighting their prospective application in minimising overuse of antibiotics to help combat antibiotic resistance.

AIM

The aim of this study is to assess the *in vitro* antimicrobial activity of several EOs against Gram-negative and Gram-positive bacteria.

METHODS

Disc Diffusion Assay

The disc diffusion method used in this study was adapted from the Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing (AST) Standards. EOs used included bergamot, cinnamon (leaf), clove, grapefruit, lavender, lemon, lemongrass, lime, manuka, orange (sweet), rose geranium, rosemary, rosewood, tea tree and thyme. Briefly, 10µL of EO was used to saturate 6mm filter paper discs (Whatman, Sigma Alrdich, UK), and placed onto Mueller-Hinton agar (MHA) plates (Oxoid, Hampshire, UK) previously inoculated with 100µL of approximately 10^6 CFU/mL of the bacteria tested (see Table 1). Gentamicin (30µg/disk) was used as a positive control and blank discs used as a negative control. Plates were incubated at 37°C for 24 h and inhibitory zones measured.

Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC)

From the preliminary disc diffusion assay, cinnamon, clove, lemongrass, manuka, rose geranium, rosemary, rosewood, tea tree and thyme were taken forward for MIC/MBC testing.

A 16% stock of EO was made using TSB with 10% DMSO. This stock was then serially diluted in TSB to create a range of concentrations from 0.0015% to 8% (v/v). Diluted EOs were added to wells of a 96-well plate (100 µl) along with 100 µl of bacterial culture (approx. $5x10^5$ CFU/mL), creating final EO concentrations of 0.0007% to 4% (v/v). The 96-well plate was incubated at 37°C for 24 h and then inspected to identify the MIC.

The MIC well and subsequent concentrations were then plated onto MHA, using a 5 µl spot inoculation method, incubated for 24 h at 37°C and then inspected to identify the MBC. MIC and MBC values are the result of 12 replicates.

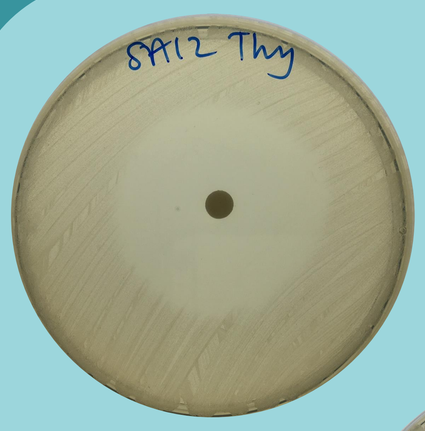

Figure 1 (left) – *Staphylococcus aureus* (NCTC 12981) treated with thyme EO using disc diffusion method

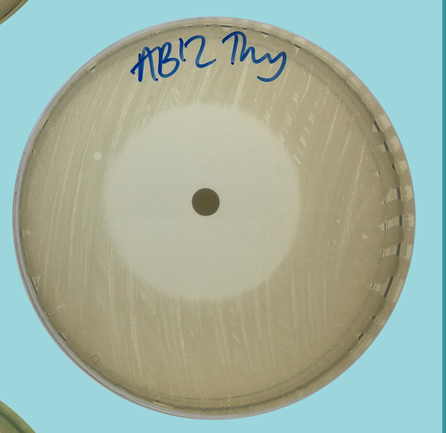

Figure 2 (right) – *Acinetobacter baumannii* (NCTC 12156) treated with thyme EO using disc diffusion method

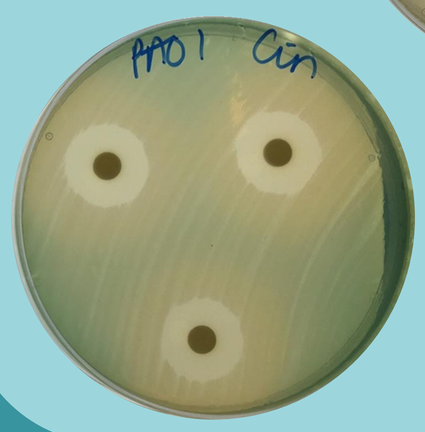

Figure 3 (left) – *Pseudomonas aeruginosa* (PAO1) treated with cinnamon (leaf) EO using disc diffusion method

RESULTS & DISCUSSION

Figures 1-3 show examples of results seen using EOs in the disc diffusion assay. From this preliminary testing it was found that several oils presented with high inhibitory potential and/or broad spectrum activity. Cinnamon, thyme, clove and lemongrass were amongst those that surpassed zones shown by the gentamicin against most, if not all, organisms.

MIC and MBC testing results (Table 1) show that the majority of the oils were able to inhibit at concentrations less than 4%. Manuka, rose geranium, rosemary and tea tree were the less effective oils, whilst cinnamon, clove, thyme and lemongrass remained the strongest. Of all the EOs tested, only cinnamon was able to inhibit and kill both *Pseudomonas* spp.

Table 1 - The antimicrobial activity of 9 EOs, using the broth microdilution method. Values are minimum inhibitory concentration and minimum bactericidal concentration (% v/v) of 12 replicates (4 independent experiments with 3 replicates each). Most successful EOs starred (★) and results >4% v/v highlighted red.

	Essential Oil	*E. coli* B ATCC 23848		*E. coli* NCTC 9001		*S. aureus* ATCC 6358		*S. aureus* NCTC 12981		Hospital Acquired MRSA isolate		MSSA NCTC 13297		*P. aeruginosa* PAO1		*P. aeruginosa* NCTC 8505		*A. baumannii* NCTC 12156		*A. baumannii* ATCC 17978	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
★	Cinnamon (leaf)	0.03	0.125	0.06	0.125	0.06	0.125	0.03	0.06	0.015	0.015	0.03	0.125	0.125	0.125	0.25	0.25	0.03	0.125	0.06	0.125
★	Clove	0.125	0.125	0.125	0.125	0.125	0.25	0.125	0.25	0.25	0.25	0.25	0.25	>4.0	>4.0	>4.0	>4.0	0.125	0.125	0.125	0.125
★	Lemongrass	0.25	0.25	1.0	1.0	0.25	0.25	0.25	0.25	0.12	0.25	0.25	0.25	>4.0	>4.0	>4.0	>4.0	1.0	1.0	0.5	1.0
	Manuka	>4.0	>4.0	>4.0	>4.0	0.06	>4.0	0.06	>4.0	0.06	>4.0	0.06	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0
	Rose Geranium	1.0	2.0	>4.0	>4.0	0.5	1.0	1.0	2.0	1.0	2.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	2.0	4.0	>4.0	>4.0
	Rosemary	2.0	2.0	4.0	4.0	2.0	>4.0	2.0	>4.0	>4.0	>4.0	2.0	>4.0	>4.0	>4.0	>4.0	>4.0	2.0	2.0	>4.0	>4.0
	Rosewood	0.5	1.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	0.5	1.0	1.0	1.0
	Tea tree	1.0	1.0	1.0	1.0	2.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	4.0	4.0	2.0	2.0
★	Thyme	0.125	0.25	0.25	0.25	0.125	0.50	0.25	0.25	0.25	0.50	0.25	0.50	>4.0	>4.0	>4.0	>4.0	0.125	0.125	0.25	0.25

CONCLUSION

As can be seen from the MIC/MBC values identified in this study there is great potential for the application of these EOs to inhibit or kill pathogenic bacteria. EOs showing the most promise from this study include cinnamon leaf, clove, thyme and lemongrass. Cinnamon leaf EO in particular has proven to be relatively broad acting, efficient at inhibiting and killing all of the organisms at concentrations <0.25%, and was the only EO in this study capable of affecting *Pseudomonas* PAO1 and NCTC 8505 at concentrations <4% (v/v).

Further studies can now be carried out to look at the interactions of these oils against target bacteria and to investigate their possible applications in the environment.

REFERENCES

Clinical and Laboratory Standards Institute (CLSI), (2012). *Performance Standards of or Antimicrobial Disk Susceptibility Tests: Approved Standard – Eleventh Edition.* CLSI document M02-A11. Pennsylvania, USA.