

ABSTRACT

Essential oils are not only used singularly but have been used in combination for many years. There is, however, very little scientific evidence to support the claims made for combined antimicrobial efficacy. With this in mind, a study was designed to investigate the antimicrobial activity of Lavender (*Lavandula angustifolia*) essential oil, in combination with other essential oils with antimicrobial relevance.

The micro-dilution minimum inhibitory concentration (MIC) assay was undertaken, whereby the fractional inhibitory concentration (Σ FIC) was calculated for 54 oil combinations. When lavender oil was assayed in 1:1 ratios with other oils, synergistic (23.5%), additive (52.5%), non-interactive (23.5%) and antagonistic (0.5%) interactions were observed. Four 1:1 combinations were synergistic against *Candida albicans* and *Staphylococcus aureus* (*Lavandula angustifolia* in combination with *Daucus carota* (Σ FIC 0.50 and 0.50); *Juniperus virginiana* (Σ FIC 0.50 and 0.50); *Cinnamomum zeylanicum* (Σ FIC 0.40 and 0.50) and *Citrus sinensis* (Σ FIC 0.42 and 0.38)). In order to understand the antimicrobial potential of these synergistic essential oil combinations, further antimicrobial analysis was undertaken whereby the oils were placed in varying ratios. Two of the four combinations (*Lavandula angustifolia* in combination with either *Cinnamomum zeylanicum* or *Citrus sinensis*), were identified as the most promising, demonstrating synergy at varying ratios, and thus the major chemical constituents of the essential oils were investigated further.

The major chemical constituents identified in *Lavandula angustifolia* (GC-MS) were linalyl acetate (36.7%), linalool (31.4%) and terpinen-4-ol (14.9%). The GC-MS profiles for all other oils in the study were also confirmed. The major chemical constituents of the most promising essential oil combinations were investigated in equal and varying ratios to determine the effect of chemistry on antimicrobial outcome. When one of the major essential oil constituents (linalyl acetate) of *Lavandula angustifolia* was combined with limonene found in *Citrus sinensis*, synergistic interactions were noted for all nine combinations against *C. albicans*; including the ratio at which the two major constituents would be mixed should the two oils be combined.

Lavandula angustifolia essential oil was placed in combination with four conventional antimicrobial agents (ciprofloxacin, chloramphenicol, fusidic acid and nystatin) to determine which of these agents in combination with *Lavandula angustifolia* would demonstrate the best antimicrobial effect. Synergy was determined for *Lavandula angustifolia* in combination with ciprofloxacin against *S. aureus* (Σ FIC of 0.49) and *Lavandula angustifolia* in combination with chloramphenicol against *P. aeruginosa* (Σ FIC of 0.29). No antagonism was noted for the combinations investigated. When placed in variable ratios it was identified that *Lavandula angustifolia* provided the pivotal role in the synergistic interactions observed against *C. albicans* and *S. aureus*, with ratios higher in *Lavandula angustifolia* essential oil concentration showing considerably better antimicrobial effects.

In order to determine the antimicrobial effects of *Lavandula angustifolia* in triple essential oil combinations, the method of MODDE[®] Design of Experiments was employed. The Design of Experiments (MODDE 9.1[®]) software identified that *Lavandula angustifolia* (from the combination of *Lavandula angustifolia*: *Citrus sinensis*: *Cedrus atlantica*) and *Thymus vulgaris* (from the combination of *Lavandula angustifolia*: *Daucus carota*: *Thymus vulgaris*) were the essential oils with the greater antimicrobial effect in the combinations analysed.

Lavender remains one of the most sought after essential oils. This comprehensive study on the antimicrobial effects of Lavender (*Lavandula angustifolia*) combinations demonstrates promising *in vitro* effects and lends some credibility for combined use in aromatherapy for the treatment of infections