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# The prediction of synergy in essential oil combinations used in the treatment of respiratory infections through experimental design and chemometric modeling

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**2024**
https://hdl.handle.net/10539/37604

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# Abstract

The therapeutic use of essential oils, or aromatherapy, is a popular practice of complementary and alternate medicine for the treatment and management of respiratory infections. Essential oils are most often applied topically via massage or via inhalation. Fifty-five essential oils and 369 combinations were identified for the management of respiratory infections. As scientific evidence supporting these indications was lacking, this study intended to explore this avenue of research.

The minimum inhibitory concentration (MIC) was recorded for each identified essential oil against nine test organisms (*Staphylococcus aureus* ATCC 25924, *Streptococcus agalactiae* ATCC 55618, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus* pyogenes ATCC 12344, *Haemophilus influenzae* ATCC 19418, *Klebsiella pneumoniae* ATCC 13883, *Moraxella catarrhalis* ATCC 23246, *Mycobacterium smegmatis* ATCC 19420 and *Cryptococcus neoformans* ATCC 14116). Gas chromatography coupled to mass spectrometry (GC-MS) was utilised to confirm purity of the essential oils and to identify the chemical profiles. The data from the GC-MS and antimicrobial studies was then analysed using chemometrics, a multivariate tool used to determine structure-activity relationships. Orthogonal projections to latent structure models were created and the compounds responsible for antimicrobial activity identified. The antimicrobial and toxic effects of the essential oils were then studied in 1:1 combinations using the MIC and brine shrimp lethality assay (BSLA), respectively. The interactive profiles of the combinations (antimicrobial and toxicity) were determined by calculating the fractional inhibitory concentration (ΣFIC) where either synergy, additivity, indifference or antagonism could be identified. Forty-nine essential oils and twenty-four essential oil combinations (1:1) were studied further for anti-inflammatory effects due to the favorable results of these oils in the MIC assay and BSLA. The anti-inflammatory activity was assessed by measuring the inhibition of nitric oxide (NO), an inflammatory mediator, production in LPS-activated RAW 264.7 macrophages. These oils were further investigated for potentially toxic effects using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) against human lung cell lines (A549). Based on the results of the anti-inflammatory and cell viability assessment against A549 cell lines, five essential oil combinations were found to be promising in equal combinations. These five combinations were then studied further by means of variable ratios using MIC analysis against the nine micro-

organisms associated with the respiratory tract. The variable ratios were plotted on isobolograms and then expanded where the MIC results were assessed using the synergy prediction software, SynergyFinder. The results of the isobolograms were compared to the SynergyFinder plots and an optimal blend of essential oils determined. The optimum blend of essential oils was then formulated into a nanoemulsion using the self-emulsification technique. The antimicrobial potential of the nanoemulsed essential oil formulation was validated by means of MIC analysis against the same nine micro-organisms and the results of the formulation compared to those of the pure essential oils in optimal ratios.

This study confirmed the antimicrobial potential of essential oils, with noteworthy inhibition observed for 31.9% of essential oils against all the pathogens tested. *Amyris balsamifera* L. (amyris), *Coriandrum sativum* L. (coriander) and *Cinnamomum zeylanicum* Blume (cinnamon) showed the broadest spectrum of activity. Computational software was applied to define the chemistry responsible for individual antimicrobial effect. Eugenol was identified as the most frequently reoccurring biomarker that contributed to broad-spectrum noteworthy antimicrobial activity of essential oils; while linalyl acetate, α-pinene and β-pinene were frequently responsible for poor antimicrobial activity. The chemometric tool was assessed for prediction accuracy by means of antimicrobial validation. The prediction accuracy relating to the chemometric outcomes of this study was 92.3% for active biomarker predictions and 61.9% for inactive biomarker predictions. Following the analysis of individual essential oils, combinations were assessed for antimicrobial potential in 1:1 and varying ratios. The prediction tool, SynergyFinder was implemented and compared to isobolograms generated for essential oil blends. Prediction accuracy for synergy by SynergyFinder was on average 34% across all nine ratios studied. This low outcome is primarily due to differences in mathematical theory applied to the definition of synergy between the isobologram and SynergyFinder. An optimal blend of essential oil was identified. This blend included the *Hyssopus officinalis* var. *angustifolius* (M.Bieb.) Benth. (hyssop) in combination with *Rosmarinus officinalis* var. *angustifolius* (Mill.) DC. (rosemary) essential oil in blends of 49.57% of *H. officinalis* to 50.43% of *R. officinalis.* This blend of essential oil was developed into a pharmaceutical nanoemulsion formulation and assessed against the same panel of pathogens. The formulation provided a six-fold enhanced antimicrobial effect as compared to the neat essential oil blend.

Essential oils, as investigated here, display antimicrobial potential alone and in combination against pathogens of the respiratory tract. Chemometric analysis was proven to be an effective tool for identifying active compounds that could potentially become novel lead compound for new antimicrobials. The blended essential oils further demonstrated potential for holistic treatment effects, proving anti-inflammatory and non-toxic activity when equally blended. It was further determined that the SynergyFinder prediction software, critical in preclinical formulation design, provides valuable supporting tools to isobolograms when optimizing essential oil combinations for antimicrobial effect. This study has also gone on to show the opportunity to combine essential oils into novel pharmaceutical formulations for enhanced antimicrobial effects. Therefore, this study has been majorly successful in identifying unique resources for antimicrobial optimization of essential oils and creates an exciting starting point for future research in this field.