Mixture design as a tool for optimization of antimicrobial activity of selected essential oils

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A natural alternative to chemically obtained food additives is the use of **essential oils (volatile oils)** whose biological activities, including antimicrobial and antioxidant properties have been confirmed for many of them and make them suitable for food preservation and other applications.
The aim of the work

The study aimed to use a statistical method of mixture design to optimize the antimicrobial activity of Tea Tree (Melaleuca alternifolia), Rosewood (Aniba roseeodora), and Lavender (Lavandula hybrida) essential oils against E. coli PCM 2057, L. monocytogenes PCM 2191, and R. mucilaginosa EPSC001.
Tea tree, rosewood, and lavender essential oils (Bianca Cosmetics Lab, Cegłów, Poland) were purchased in a local pharmacy in Warsaw (Poland).

In the study following microorganisms were used: R. mucilaginosa EPSC001 (WULS, Poland), E. coli PCM 2057, and L. monocytogenes PCM 2191 purchased from the Polish Collection of Microorganisms (PCM) of Institute of Immunology and Experimental Therapy PAS (Wroclaw, Poland).

- Determination of Total Phenolic Content and Antioxidant Activity by the DPPH· and CUPRAC Methods
- Mixture design - Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA)
- Evaluation of Antimicrobial Properties of Essential Oils by the Disc Diffusion Method
- Statistical analysis
Table 1. Simplex-Lattice Design, experimentally measured inhibition zone diameters, approximated values and residues for 3 tested microorganisms.

| Exp. No. | Essential Oil | E. coli PCM 2057 | L. monocytogenes PCM 2191 | R. mucilaginosa EPSC001 |
|----------|---------------|------------------|---------------------------|-------------------------|
|          |               | Measured | Approx.| Residues | Measured | Approx.| Residues | Measured | Approx.| Residues |
| 1        | A\(^1\) 1.00  B 0.00  C 0.00 | 11.33 | 10.85 | 0.48 | 11.33 | 11.34 | -0.01 | 11.00 | 11.31 | -0.31 |
| 2        | A\(^1\) 0.00  B 1.00  C 0.00 | 19.00 | 19.29 | -0.29 | 18.33 | 17.98 | 0.35 | 17.33 | 16.82 | 0.51 |
| 3        | A\(^1\) 0.00  B 0.00  C 1.00 | 13.33 | 11.96 | 1.37 | 12.33 | 12.43 | -0.10 | 11.33 | 11.31 | 0.02 |
| 4        | A\(^1\) 0.50  B 0.50  C 0.00 | 16.33 | 15.07 | 1.26 | 11.33 | 10.82 | 0.51 | 15.33 | 15.13 | 0.20 |
| 5        | A\(^1\) 0.50  B 0.00  C 0.50 | 11.33 | 11.40 | -0.07 | 9.33 | 9.27 | 0.06 | 14.00 | 14.28 | -0.28 |
| 6        | A\(^1\) 0.00  B 0.50  C 0.50 | 17.33 | 15.63 | 1.70 | 15.33 | 14.91 | 0.42 | 16.67 | 16.13 | 0.54 |
| 7        | A\(^1\) 0.67  B 0.17  C 0.17 | 12.33 | 12.44 | -0.11 | 9.33 | 9.73 | -0.40 | 11.33 | 10.64 | 0.69 |
| 8        | A\(^1\) 0.17  B 0.67  C 0.17 | 16.33 | 16.66 | -0.33 | 12.33 | 13.82 | -1.49 | 11.33 | 13.10 | -1.77 |
| 9        | A\(^1\) 0.17  B 0.17  C 0.67 | 10.67 | 13.00 | -2.33 | 11.33 | 11.45 | -0.12 | 10.67 | 10.98 | -0.31 |
| 10       | A\(^1\) 0.33  B 0.33  C 0.33 | 12.33 | 14.03 | -1.70 | 11.67 | 10.92 | 0.75 | 9.33  | 8.64  | 0.69 |

\(^1\) A – Lavender Essential Oil; B – Tea Tree Essential Oil; C – Rosewood Essential Oil
Table 2. Total phenolic content and antioxidant activity by means of the DPPH- and CUPRAC methods.

| Essential oil       | DPPH-    | Methanolic extract | CUPRAC   | TPC       |
|---------------------|----------|--------------------|----------|-----------|
|                     | AA (%)   | TEAC (µmol Trolox/g EO) | AA (%)   | TEAC (µmol Trolox/g EO) | TEAC (µmol Trolox/g EO) | mg GA/g EO |
| Tea Tree            | 12.14 ± 1.30A* | 2.22 ± 0.23A | 10.93 ± 0.24A | 2.01 ± 0.04A | 6.55 ± 0.78A | 0.59 ± 0.05A |
| Rosewood            | 7.56 ± 2.29B | 1.42 ± 0.40B | 8.29 ± 0.90B | 1.55 ± 0.16B | 1.67 ± 0.40B | 0.11 ± 0.02C |
| Lavender            | 12.60 ± 0.92A | 2.30 ± 0.16A | 11.97 ± 0.85A | 2.19 ± 0.15A | 5.99 ± 0.80A | 0.27 ± 0.05B |

Abbreviations: AA – antioxidant activity; TEAC – Trolox equivalent antioxidant capacity; EO – essential oil; GA – gallic acid; TPC – total phenolic content.

* The values with a different letter (A–C) in a column are significantly different (α = 0.05).
Table 3. ANOVA results for different statistical models for *E. coli* PCM 2057.

| Model            | SS     | df  | MS    | F      | p-value | R²     | R²_adj |
|------------------|--------|-----|-------|--------|---------|--------|--------|
| Linear           | 63.1605| 2   | 31.5802| 14.5802| 0.0032  | 0.8064 | 0.7511 |
| Quadratic        | 4.7560 | 3   | 1.5853| 0.6094 | 0.6434  | 0.8671 | 0.7011 |
| Special cubic    | 6.7985 | 1   | 6.7985| 5.6540 | 0.0978  | 0.9539 | 0.8618 |
| Cubic            | 3.2391 | 2   | 1.6195| 4.3986 | 0.3195  | 0.9953 | 0.9577 |

1 SS – sum of square; df – degrees of freedom; MS – mean of square; F – F-values; R² – coefficient of determination; R²_adj – adjusted coefficient of determination

At the level of *p* < 0.05, statistical significance was observed for:

Table 4. ANOVA results for different statistical models for *L. monocytogenes* PCM 2191.

| Model            | SS     | df  | MS    | F      | p-value | R²     | R²_adj |
|------------------|--------|-----|-------|--------|---------|--------|--------|
| Linear           | 45.4444| 2   | 22.7222| 7.5901 | 0.0177  | 0.6844 | 0.5942 |
| Quadratic        | 17.4328| 3   | 5.8109| 6.5982 | 0.0499  | 0.9469 | 0.8806 |
| Special cubic    | 0.0882 | 1   | 0.0882| 0.0771 | 0.9483  | 0.8448 |
| Cubic            | 1.2727 | 2   | 0.6364| 0.2944 | 0.7934  | 0.9674 | 0.7070 |

1 SS – sum of square; df – degrees of freedom; MS – mean of square; F – F-values; R² – coefficient of determination; R²_adj – adjusted coefficient of determination

Table 5. ANOVA results for different statistical models for *R. mucilaginosa* EPSC001.

| Model             | SS     | df  | MS    | F      | p-value | R²     | R²_adj |
|-------------------|--------|-----|-------|--------|---------|--------|--------|
| Linear            | 24.0494| 2   | 12.0247| 1.8474 | 0.2268  | 0.3455 | 0.1385 |
| Quadratic         | 3.7545 | 3   | 1.2515| 0.1197 | 0.9438  | 0.3994 | 0.0000 |
| Special cubic     | 36.8802| 1   | 36.8802| 22.4557| 0.0178  | 0.9292 | 0.7877 |
| Cubic             | 3.7239 | 2   | 1.8620| 1.5476 | 0.4942  | 0.9827 | 0.8444 |

1 SS – sum of square; df – degrees of freedom; MS – mean of square; F – F-values; R² – coefficient of determination; R²_adj – adjusted coefficient of determination

In the case of *L. monocytogenes* for further analyses, the quadratic model was chosen due to the higher R² value (0.9469) in comparison with the linear model (R² = 0.6844).
Figure 1. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a linear model for *E. coli* PCM 2057.
Figure 2. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a quadratic model for *L. monocytogenes* PCM 2191.
Figure 3. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a special cubic model for *R. mucilaginosa* EPSC001.
Conclusion

These experiments confirmed the possibility of using statistical methods, and in the current study - mixture design with the use of the simplex-lattice plan to develop an optimal essential oils blend with high antimicrobial activity.

A natural progression of this work is to analyze the compositions of the essential oils.

Further research should focus also on determining possible synergistic effects of tested volatile oils, as well as on establishing the mechanisms of action of compounds included in obtained mixtures on microorganisms.
References

1. Valdivieso-Ugarte, M.; Gomez-Llorente, C.; Plaza-Díaz, J.; Gil, Á. Antimicrobial, Antioxidant, and Immunomodulatory Properties of Essential Oils: A Systematic Review. *Nutrients* **2019**, *11*, 2786. [https://doi.org/10.3390/nu11112786](https://doi.org/10.3390/nu11112786)

2. Rybak, K.; Wiktor, A.; Witrowa-Rajchert, D.; Parniakov, O.; Nowacka, M. The Quality of Red Bell Pepper Subjected to Freeze-Drying Preceded by Traditional and Novel Pretreatment. *Foods* **2021**, *10*, 226. [https://doi.org/10.3390/foods10020226](https://doi.org/10.3390/foods10020226)

3. Zieniuk, B.; Groborz, K.; Wołoszynowska, M.; Ratusz, K.; Białecka-Florjańczyk, E.; Fabiszewska, A. Enzymatic Synthesis of Lipophilic Esters of Phenolic Compounds, Evaluation of Their Antioxidant Activity and Effect on the Oxidative Stability of Selected Oils. *Biomolecules* **2021**, *11*, 314. [https://doi.org/10.3390/biom11020314](https://doi.org/10.3390/biom11020314)

4. Garzoli, S.; Turchetti, G.; Giacomello, P.; Tiezzi, A.; Laghezza Masci, V.; Ovidi, E. Liquid and Vapour Phase of Lavandin (*Lavandula × intermedia*) Essential Oil: Chemical Composition and Antimicrobial Activity. *Molecules* **2019**, *24*, 2701. [https://doi.org/10.3390/molecules24152701](https://doi.org/10.3390/molecules24152701)

5. Teles, A.M.; Silva-Silva, J.V.; Fernandes, J.M.P.; Calabrese, K.d.S.; Abreu-Silva, A.L.; Marinho, S.C.; Mouchrek, A.N.; Filho, V.E.M.; Almeida-Souza, F. *Aniba rosaedora* (Var. amazonica Bucke) Essential Oil: Chemical Composition, Antibacterial, Antioxidant and Antitrypanosomal Activity. *Antibiotics* **2021**, *10*, 24. [https://doi.org/10.3390/antibiotics10010024](https://doi.org/10.3390/antibiotics10010024)

6. Bajalan, I.; Rouzbahani, R.; Pirbalouti, A.G.; Maggi, F. Chemical Composition and Antibacterial Activity of Iranian *Lavandula × hybrida*. *Chem. Biodivers.* **2017**, *14*(7), e1700064. [https://doi.org/10.1002/cbdv.201700064](https://doi.org/10.1002/cbdv.201700064)

7. Liao, M.; Xiao, J.J.; Zhou, L.J.; Yao, X.; Tang, F.; Hua, R.M.; Wu, X.W.; Cao, H.Q. Chemical composition, insecticidal and biochemical effects of *Melaleuca alternifolia* essential oil on the *Helicoverpa armigera*. *J. Appl. Entomol.* **2017**, *9*, 721-728. [https://doi.org/10.1111/jen.12397](https://doi.org/10.1111/jen.12397)

8. Baj, T.; Baryluk, A.; Sieniawska, E. Application of mixture design for optimum antioxidant activity of mixtures of essential oils from *Ocimum basilicum* L., *Origanum majorana* L. and *Rosmarinus officinalis* L. *Ind Crops Prod* **2018**, *115*, 52-61. [https://doi.org/10.1016/j.indcrop.2018.02.006](https://doi.org/10.1016/j.indcrop.2018.02.006)

9. Crespo, Y.A.; Sanchez, L.R.B.; Quintana, Y.G.; Cabrera, A.S.T.; del Sol, A.B.; Mayanchar, D.M.G. Evaluation of the synergistic effects of antioxidant activity on mixtures of the essential oil from *Apium graveolens* L., *Thymus vulgaris* L. and *Coriandrum sativum* L. using simplex-lattice design. *Heliyon* **2019**, *5*(6), e01942. [https://doi.org/10.1016/j.heliyon.2019.e01942](https://doi.org/10.1016/j.heliyon.2019.e01942)

10. Fadil, M.; Fikri-Benbrahim, K.; Rachiq, S.; Ihssane, B.; Lebrazi, S.; Chraibi, M.; Haloui, T.; Farah, A. Combined treatment of *Thymus vulgaris* L., *Rosmarinus officinalis* L. and *Myrtus communis* L. essential oils against *Salmonella typhimurium*: Optimization of antibacterial activity by mixture design methodology. *Eur. J. Pharm. Biopharm.* **2018**, *126*, 211-220. [https://doi.org/10.1016/j.ejpb.2017.06.002](https://doi.org/10.1016/j.ejpb.2017.06.002)
Thank you for your attention!