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

Bartłomiej Zieniuk[1], and Anna Bętkowska[2]

[1]Department of Chemistry, Institute of Food Sciences, Warsaw University of Life Sciences-SGGW, 159c Nowoursynowska St., 02-776 Warsaw, Poland; bartlomiej_zieniuk@sggw.edu.pl

[2]Scientific Circle of Biotechnology Students "KNBiotech", Faculty of Horticulture and Biotechnology, Warsaw University of Life Sciences-SGGW, 159 Nowoursynowska St., 02-776 Warsaw, Poland; ania.betkowska@gmail.com

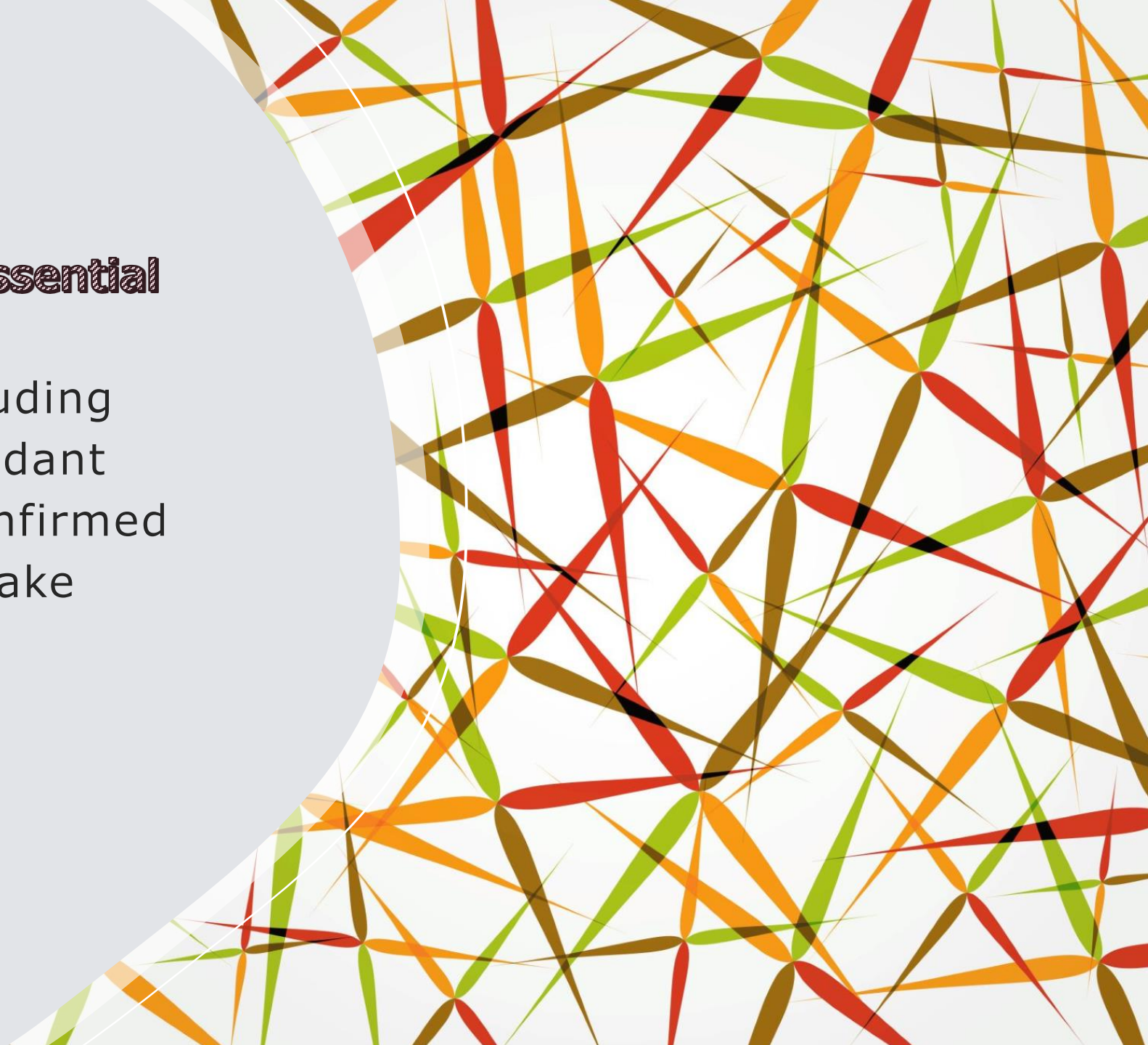
Foods 2021

The 2nd International Electronic Conference on Foods

Future Foods and Food Technologies for a Sustainable World

15–30 OCTOBER 2021 | ONLINE

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 rosaeodora*), and **Lavender** (*Lavandula hybrida*) essential oils against ***E. coli*** PCM 2057, ***L. monocytogenes*** PCM 2191, and ***R. mucilaginosa*** EPSC001.

MATERIALS AND METHODS

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 (Wrocław, 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		
				Inhibition Zone Diameters								
	A[1]	B	C	Measured	Approx.	Residues	Measured	Approx.	Residues	Measured	Approx.	Residues
1	1.00	0.00	0.00	**11.33**	10.85	0.48	**11.33**	11.34	-0.01	**11.00**	11.31	-0.31
2	0.00	1.00	0.00	**19.00**	19.29	-0.29	**18.33**	17.98	0.35	**17.33**	16.82	0.51
3	0.00	0.00	1.00	**13.33**	11.96	1.37	**12.33**	12.43	-0.10	**11.33**	11.31	0.02
4	0.50	0.50	0.00	**16.33**	15.07	1.26	**11.33**	10.82	0.51	**15.33**	15.13	0.20
5	0.50	0.00	0.50	**11.33**	11.40	-0.07	**9.33**	9.27	0.06	**14.00**	14.28	-0.28
6	0.00	0.50	0.50	**17.33**	15.63	1.70	**15.33**	14.91	0.42	**16.67**	16.13	0.54
7	0.67	0.17	0.17	**12.33**	12.44	-0.11	**9.33**	9.73	-0.40	**11.33**	10.64	0.69
8	0.17	0.67	0.17	**16.33**	16.66	-0.33	**12.33**	13.82	-1.49	**11.33**	13.10	-1.77
9	0.17	0.17	0.67	**10.67**	13.00	-2.33	**11.33**	11.45	-0.12	**10.67**	10.98	-0.31
10	0.33	0.33	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.

	DPPH·				CUPRAC	TPC
	Essential oil		Methanolic extract			
	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 ± 1.30^{A*}	2.22 ± 0.23^{A}	10.93 ± 0.24^{A}	2.01 ± 0.04^{A}	**6.55 ± 0.78^{A}**	**0.59 ± 0.05^{A}**
Rosewood	7.56 ± 2.29^{B}	1.42 ± 0.40^{B}	8.29 ± 0.90^{B}	1.55 ± 0.16^{B}	1.67 ± 0.40^{B}	0.11 ± 0.02^{C}
Lavender	**12.60 ± 0.92^{A}**	**2.30 ± 0.16^{A}**	**11.97 ± 0.85^{A}**	**2.19 ± 0.15^{A}**	5.99 ± 0.80^{A}	0.27 ± 0.05^{B}

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 ($\alpha = 0.05$).

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

Table 3. ANOVA results for different statistical models for *E. coli* PCM 2057.

Model	SS[1]	df	MS	F	*p-value*	R^2	R^2_{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; R^2 – coefficient of determination; R^2_{adj} – adjusted coefficient of determination

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

Model	SS[1]	df	MS	F	*p-value*	R^2	R^2_{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	0.7993	0.9483	0.8448
Cubic	1.2727	2	0.6364	0.2944	0.7934	0.9674	0.7070

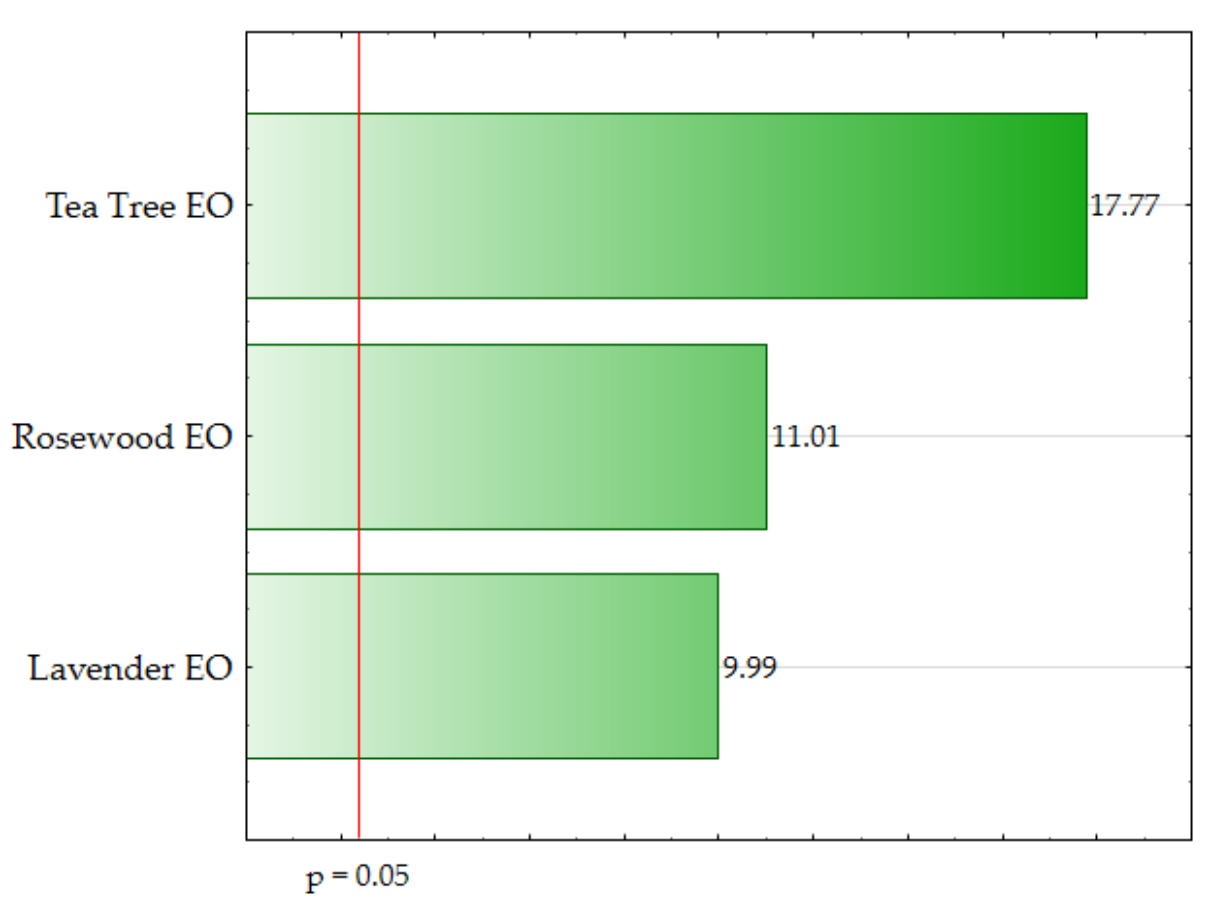
[1] SS – sum of square; df – degrees of freedom; MS – mean of square; F – F-values; R^2 – coefficient of determination; R^2_{adj} – adjusted coefficient of determination

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

Model	SS[1]	df	MS	F	*p-value*	R^2	R^2_{adj}
Linear	24.0494	2	12.0247	1.8474	0.2268	0.3455	0.1585
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; R^2 – coefficient of determination; R^2_{adj} – adjusted coefficient of determination

In the case of *L. monocytogenes* for further analyses, the quadratic model was chosen due to the higher R^2 value (0.9469) in comparison with the linear model (R^2 = 0.6844).



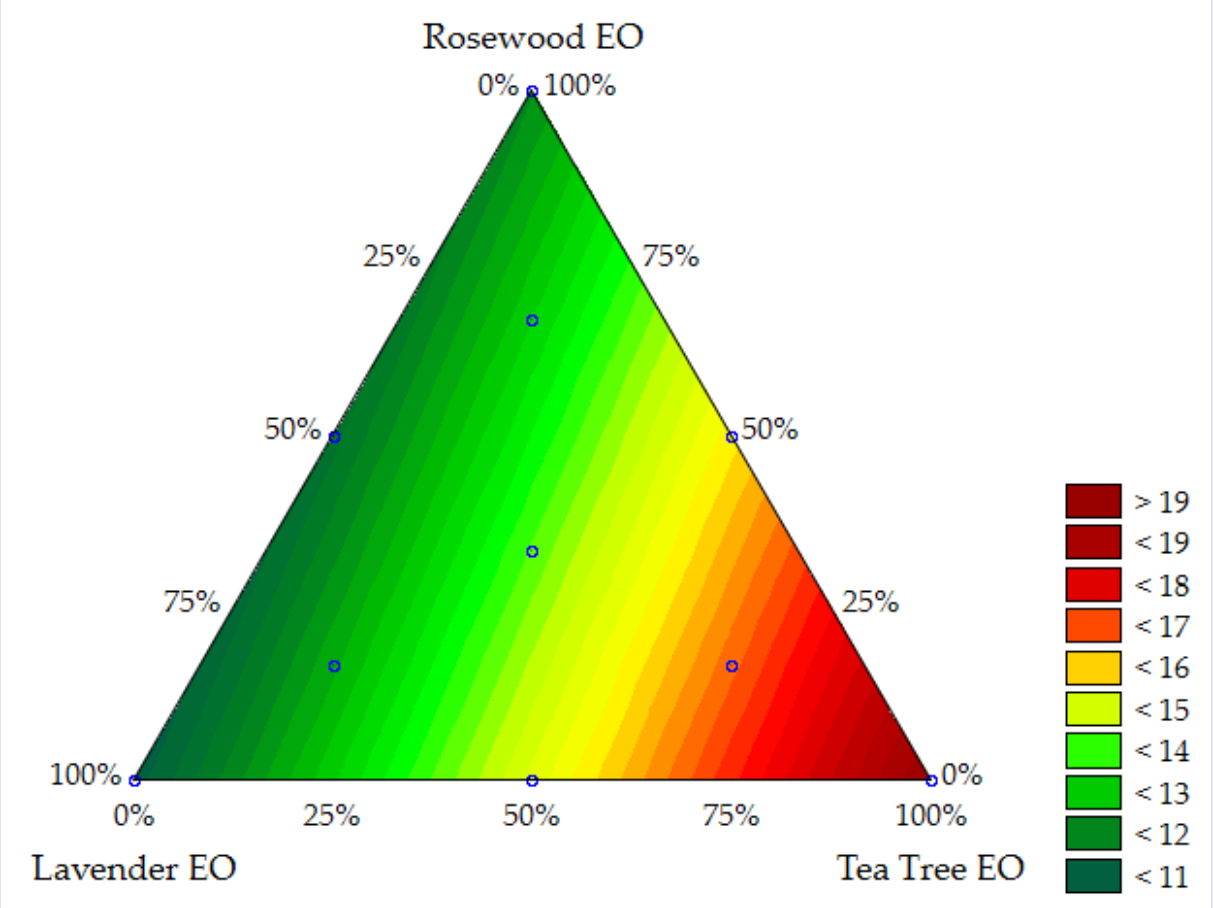
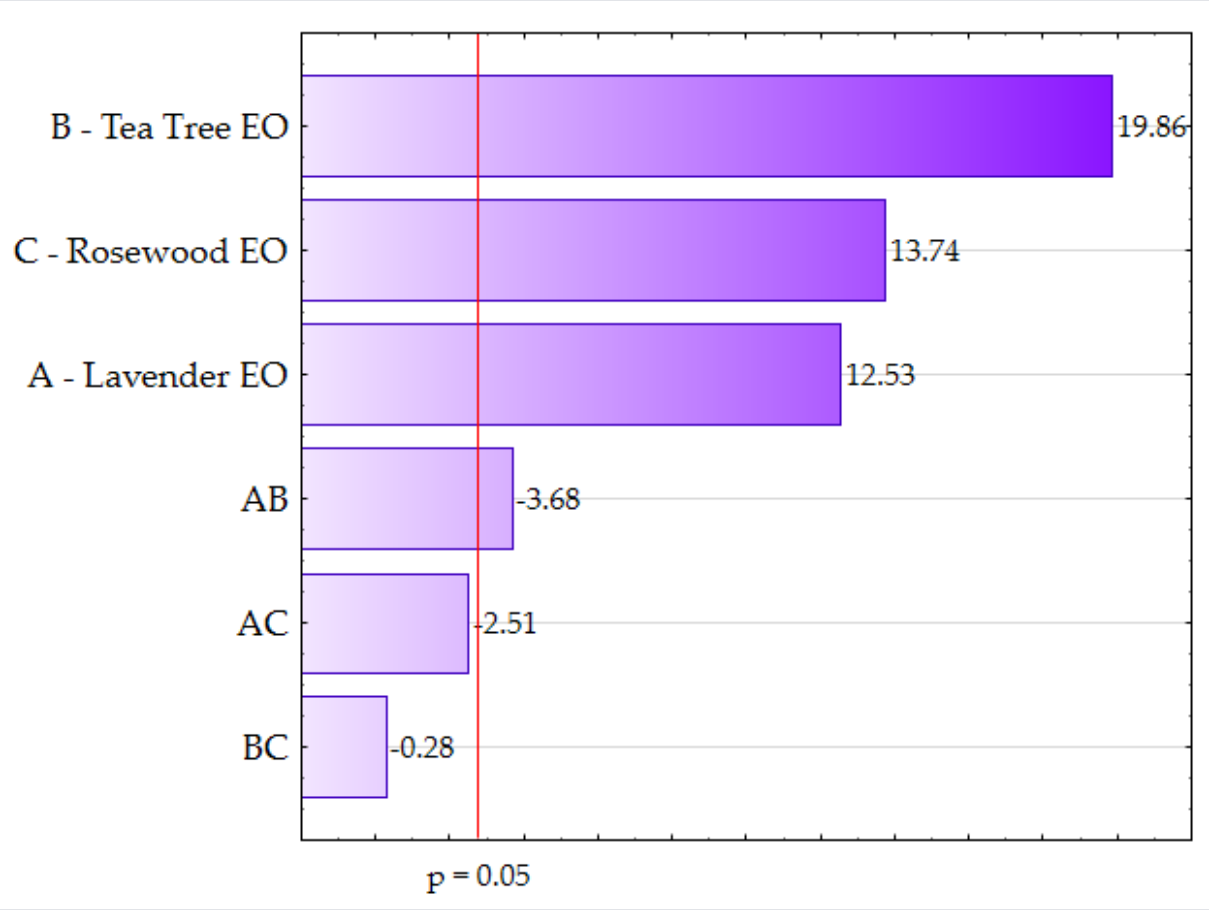


Figure 1. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a linear model for *E. coli* PCM 2057.



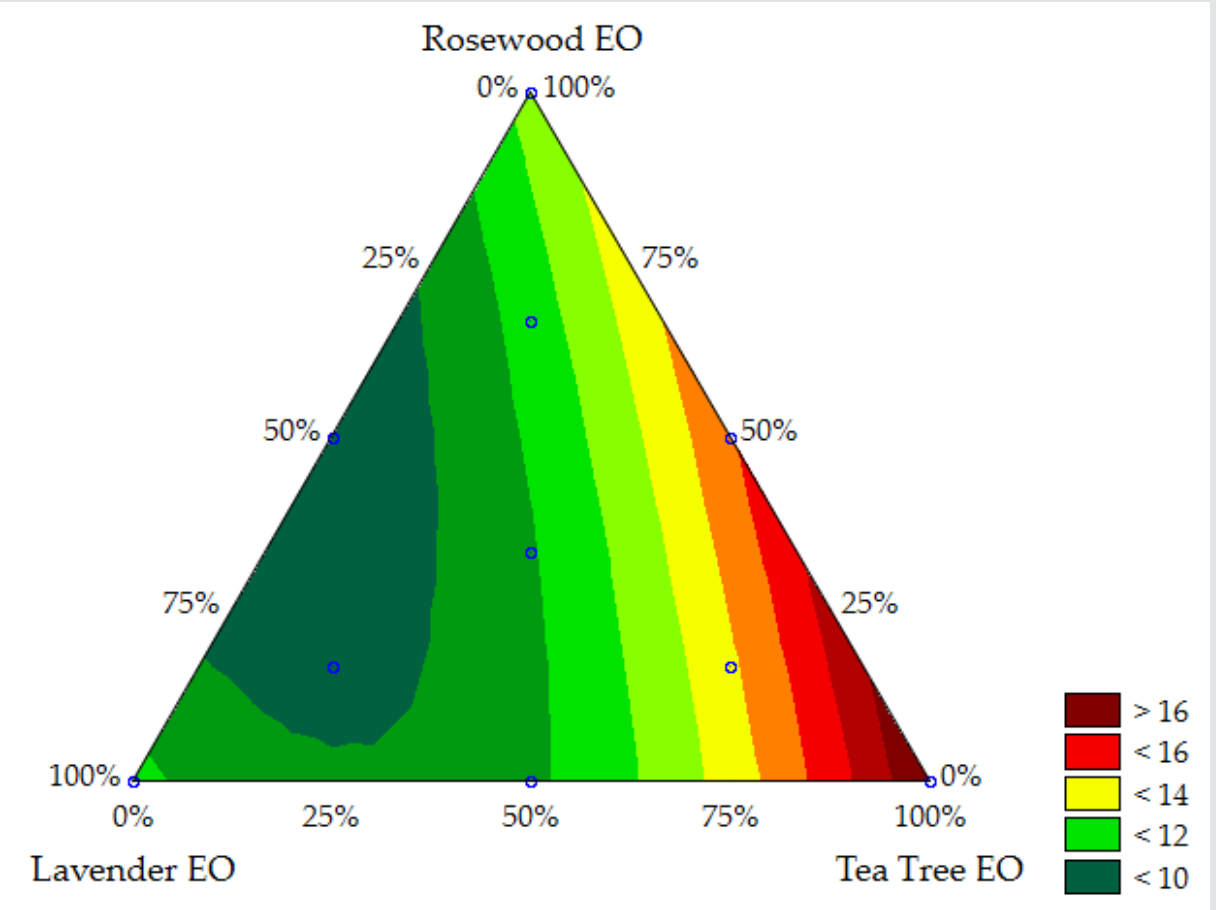
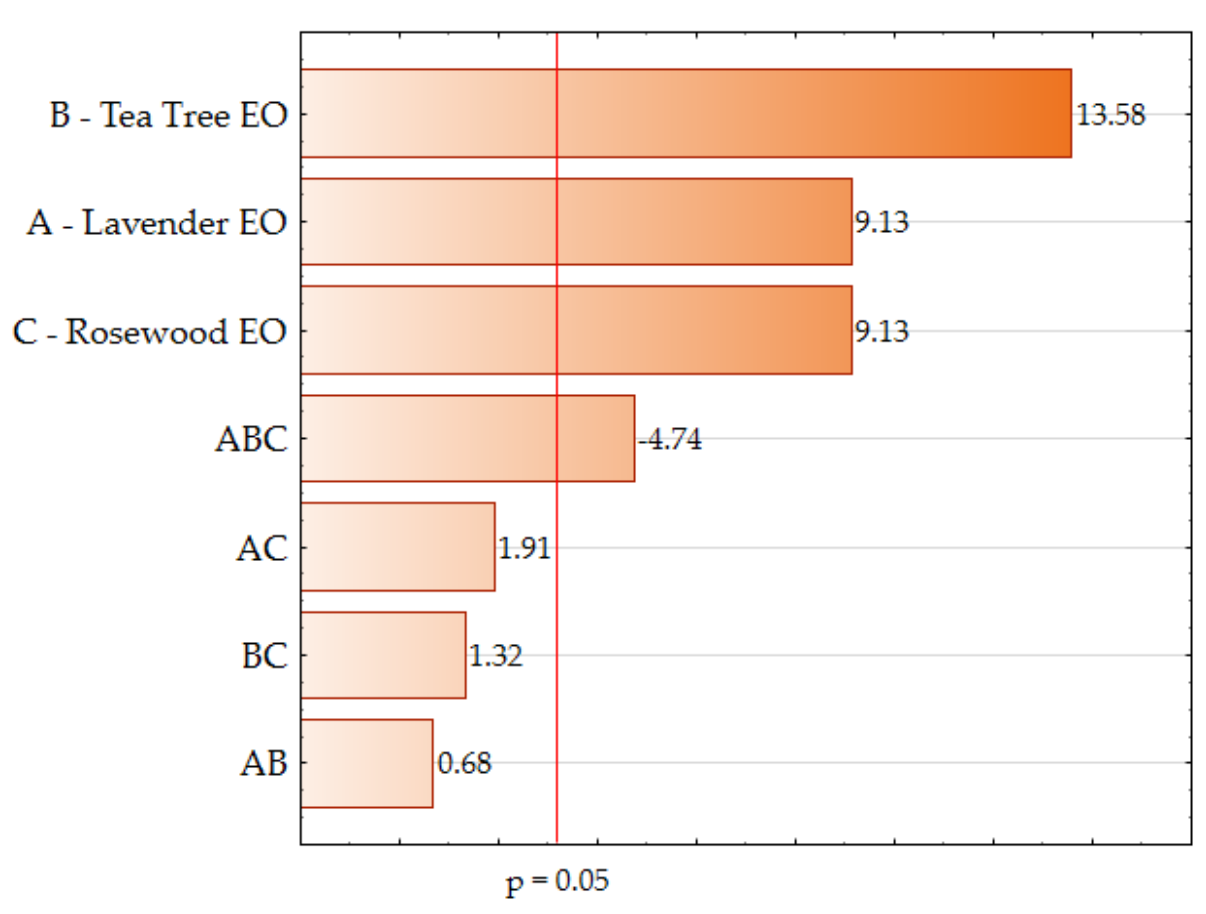


Figure 2. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a quadratic model for *L. monocytogenes* PCM 2191.



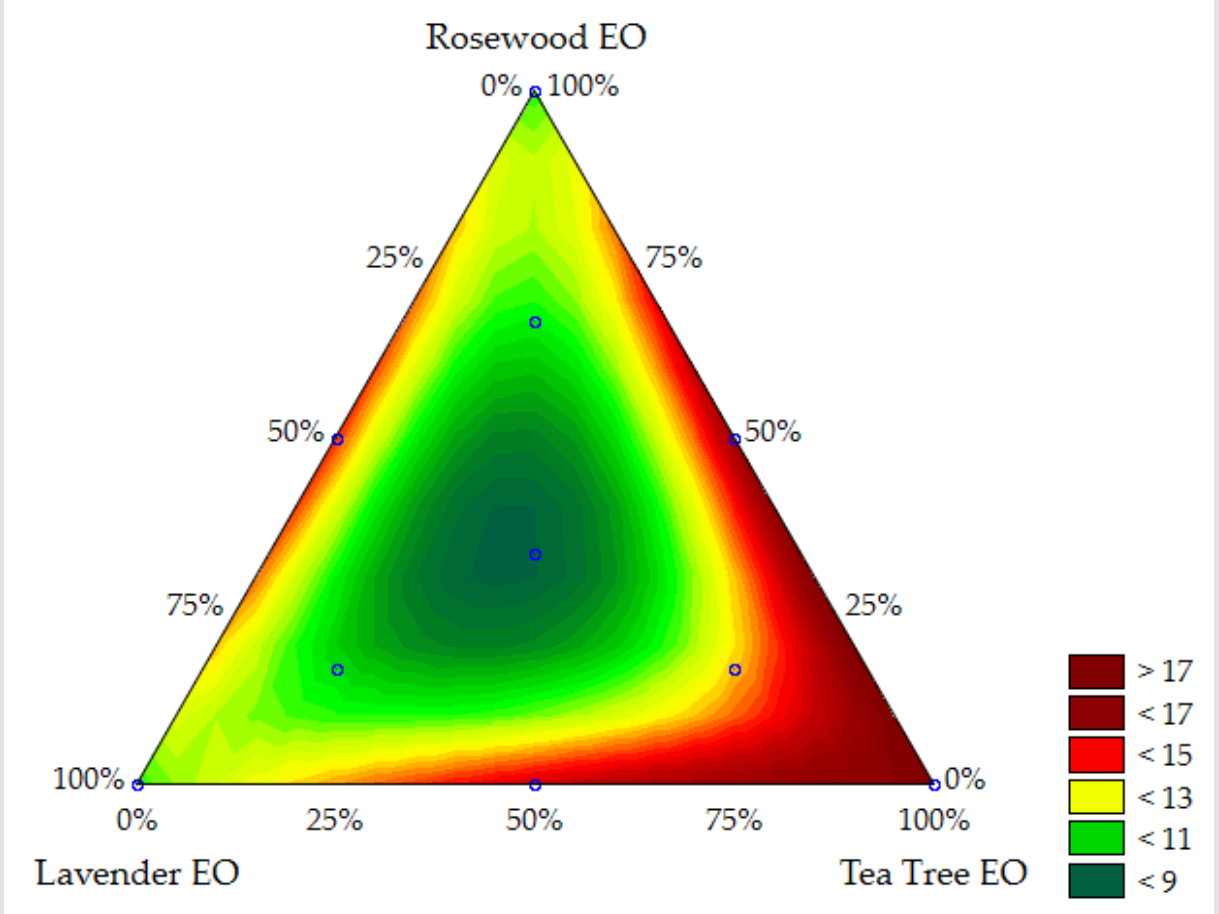


Figure 3. Optimization of antimicrobial activity by means of mixture design, presented as Pareto chart and contour plot for a special cubic model for *R. mucilaginosa* EPSC001.

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

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

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

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

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 rosaeodora* (Var. amazonica Ducke) Essential Oil: Chemical Composition, Antibacterial, Antioxidant and Antitrypanosomal Activity. *Antibiotics* **2021**, *10*, 24. 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

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

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

9. Crespo, Y.A.; Sanchez, L.R.B.; Quintana, Y.G.; Cabrera, A.S.T.; del Sol, A.B.; Mayancha, 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

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

Thank you for your attention!