

Propolis as a Cariostatic Agent in Lozenges and Impact of Storage Conditions on the Stability of Propolis

Anna Kurek-Górecka ^{1,*}, Paweł Ramos ², Małgorzata Klósek ³, Elżbieta Bobela ³, Zenon P. Czuba ³, Radosław Balwierz ⁴ and Paweł Olczyk ¹

¹ Department of Community Pharmacy, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Kasztanowa 3, 41-200 Sosnowiec, Poland; polczyk@sum.edu.pl

² Department of Biophysics, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Jedności 8, 41-200 Sosnowiec, Poland; pawelramos@sum.edu.pl


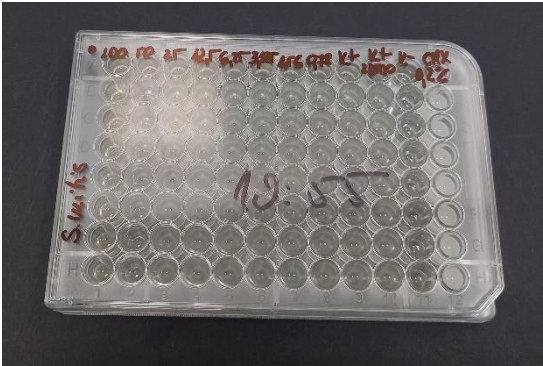

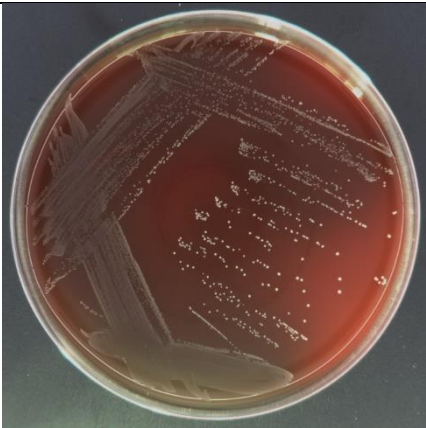

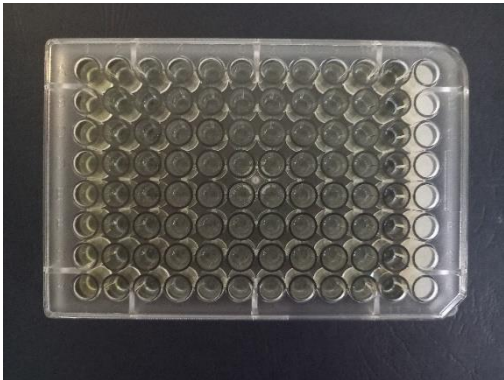
³ Department of Microbiology and Immunology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia in Katowice, Jordana 19, 41-808 Zabrze, Poland; mklosek@sum.edu.pl (M.K.); ebobela@sum.edu.pl (E.B.); zczuba@sum.edu.pl (Z.P.C.)

⁴ Institute of Chemistry, University of Opole, Oleska 48, 45-052 Opole, Poland; radoslaw.balwierz@uni.opole.pl

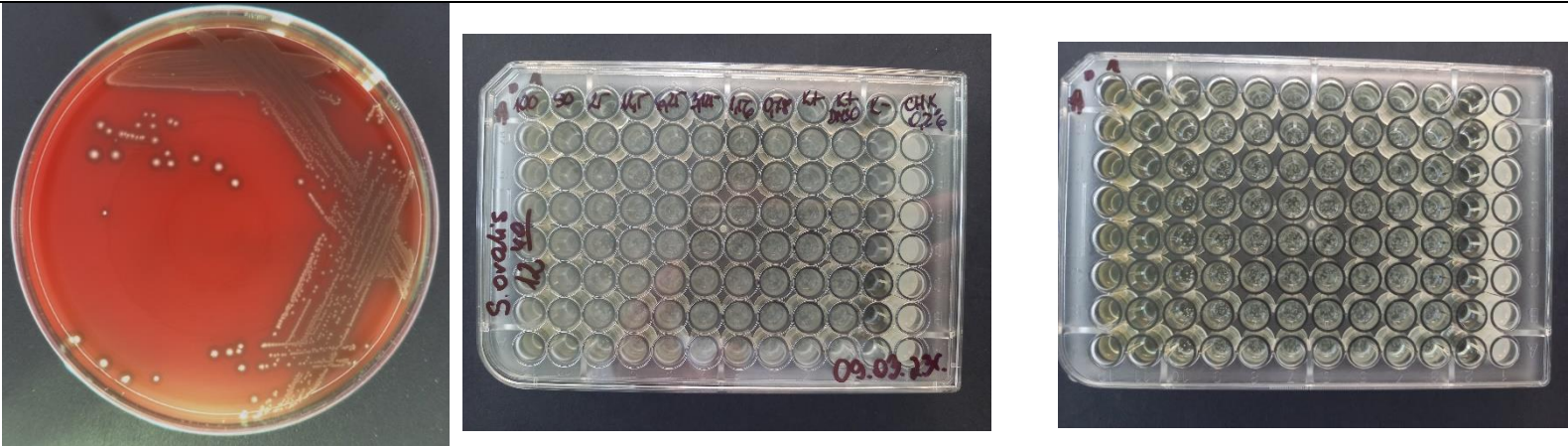
* Correspondence: akurekgorecka@sum.edu.pl

| Table of content | p. |
|--|----|
| Table S1. Photos presenting 96-well plates and plates with broth made to determine the minimum bactericidal concentration for EEP. | 2 |
| Table S2. Results of main effect of ANOVA analysis for MIC of tested EEP concentrations. | 4 |
| Table S3. Results of main effect of ANOVA analysis for MIC values of tested lozenges. | 4 |
| Table S4. Results of Fisher's LSD Test for <i>S. mitis</i> MIC values of tested lozenges. | 5 |
| Table S5. Results of Fisher's LSD Test for <i>S. mutans</i> MIC values of tested lozenges. | 6 |
| Table S6. Results of Fisher's LSD Test for <i>S. oralis</i> MIC values of tested lozenges. | 7 |
| Table S7. Results of Fisher's LSD Test for <i>S. salivarius</i> MIC values of tested lozenges. | 8 |
| Table S8. Photos presenting 96-well plates and plates with broth made to determine the minimum bactericidal concentration for prepared lozenges with EEP. | 9 |

Table S1. Photos presenting 96-well plates and plates with broth made to determine the minimum bactericidal concentration for EEP.

| Bacteria | Photos of the plate presenting strains bacteria tested | Photos of the 96-well microtiter plates used to determination of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of EEP | |
|-----------------------------|--|---|--|
| <i>Streptococcus mitis</i> |  |  |  |
| <i>Streptococcus mutans</i> |  |  |  |

Streptococcus oralis



Streptococcus salivarius

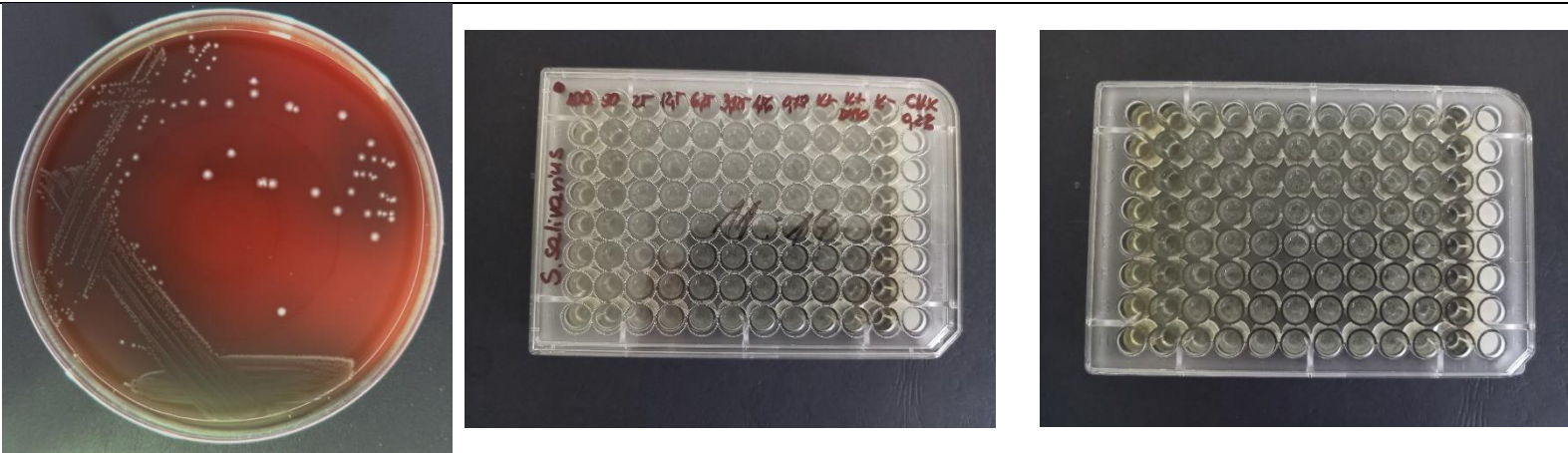


Table S2. Results of main effect of ANOVA analysis for MIC of tested EEP concentrations.

| Effect | Multivariate Tests of Significance Sigma-restricted parameterization Effective hypothesis decomposition | | | | |
|-------------|---|----------|----------|-----------|----------|
| | Test | Value | F | Effect df | Error df |
| Intercept | Wilks | 0,001428 | 14160,06 | 4 | 81,0000 |
| Formulation | Wilks | 0,000079 | 76,57 | 44 | 311,8403 |

Results marked in red are statistically significant at the level $p \leq 0.05$.

Table S3. Results of main effect of ANOVA analysis for MIC values of tested lozenges.

| Effect | Multivariate Tests of Significance0 Sigma-restricted parameterization Effective hypothesis decomposition | | | | |
|-------------|--|----------|----------|-----------|----------|
| | Test | Value | F | Effect df | Error df |
| Intercept | Wilks | 0,004078 | 4762,232 | 4 | 78,0000 |
| Formulation | Wilks | 0,003115 | 48,173 | 24 | 273,3195 |

* Results marked in red are statistically significant at the level $p \leq 0.05$.

Table S4. Results of Fisher's LSD Test for *S. mitis* MIC values of tested lozenges.

| LSD test; variable <i>S. mitis</i> | | | | | | | | |
|--|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Probabilities for Post Hoc Tests Error: Between MS = .00048. df = 49.000 | | | | | | | | |
| Cell No. | Formulation | {1} .29812 | {2} .28412 | {3} .27088 | {4} .35012 | {5} .04212 | {6} .06425 | {7} .04037 |
| 1 | PT | | 0.206348 | 0.016107 | 0.000018 | 0.000000 | 0.000000 | 0.000000 |
| 2 | PT/HT | 0.206348 | | 0.231311 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 3 | PT/UV30m | 0.016107 | 0.231311 | | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 4 | PT/UV60m | 0.000018 | 0.000000 | 0.000000 | | 0.000000 | 0.000000 | 0.000000 |
| 5 | Control (saliva & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | | 0.048454 | 0.873476 |
| 6 | Control (tablets WP & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.048454 | | 0.033782 |
| 7 | Control (saliva) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.873476 | 0.033782 | |

Results marked in red are statistically significant at the level $p \leq 0.05$.

Table S5. Results of Fisher's LSD Test for *S. mutans* MIC values of tested lozenges.

| LSD test; variable <i>S. mutans</i> | | | | | | | | |
|--|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Probabilities for Post Hoc Tests Error: Between MS = .00267. df = 49.000 | | | | | | | | |
| Cell No. | Formulation | {1} 1.3363 | {2} 1.1121 | {3} 1.0305 | {4} 1.1420 | {5} .05487 | {6} .08238 | {7} .05237 |
| 1 | PT | | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 2 | PT/HT | 0.000000 | | 0.002725 | 0.253465 | 0.000000 | 0.000000 | 0.000000 |
| 3 | PT/UV30m | 0.000000 | 0.002725 | | 0.000078 | 0.000000 | 0.000000 | 0.000000 |
| 4 | PT/UV60m | 0.000000 | 0.253465 | 0.000078 | | 0.000000 | 0.000000 | 0.000000 |
| 5 | Control (saliva & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | | 0.292681 | 0.923359 |
| 6 | Control (tablets WP & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.292681 | | 0.251511 |
| 7 | Control (saliva) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.923359 | 0.251511 | |

Results marked in red are statistically significant at the level $p \leq 0.05$.

Table S6. Results of Fisher's LSD Test for *S. oralis* MIC values of tested lozenges.

| LSD test; variable <i>S. oralis</i> | | | | | | | | |
|--|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Probabilities for Post Hoc Tests Error: Between MS = .00116. df = 49.000 | | | | | | | | |
| Cell No. | Formulation | {1} 1.2576 | {2} 1.0605 | {3} .98200 | {4} 1.1495 | {5} .05475 | {6} .11000 | {7} .05275 |
| 1 | PT | | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 2 | PT/HT | 0.000000 | | 0.000029 | 0.000004 | 0.000000 | 0.000000 | 0.000000 |
| 3 | PT/UV30m | 0.000000 | 0.000029 | | 0.000000 | 0.000000 | 0.000000 | 0.000000 |
| 4 | PT/UV60m | 0.000000 | 0.000004 | 0.000000 | | 0.000000 | 0.000000 | 0.000000 |
| 5 | Control (saliva & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | | 0.002121 | 0.906979 |
| 6 | Control (tablets WP & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.002121 | | 0.001507 |
| 7 | Control (saliva) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.906979 | 0.001507 | |

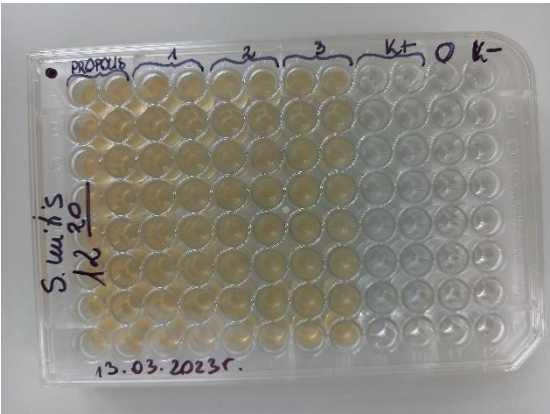
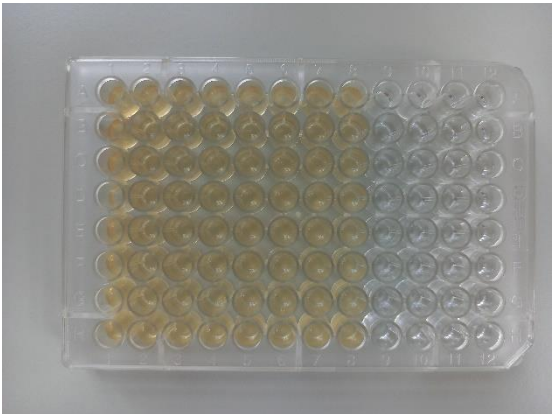
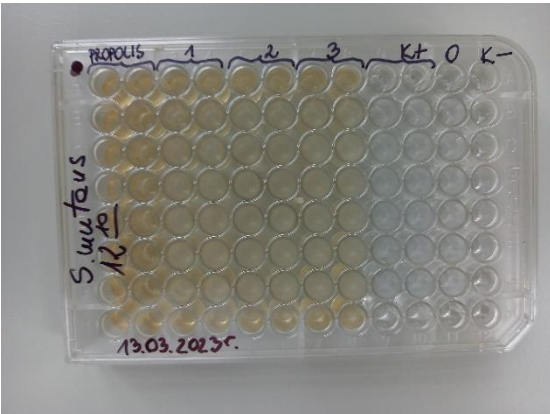
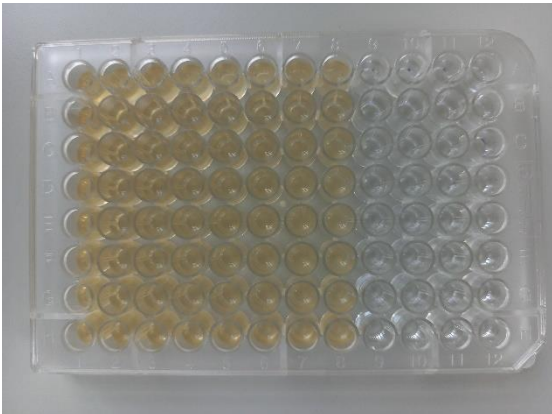
Results marked in red are statistically significant at the level $p \leq 0.05$.

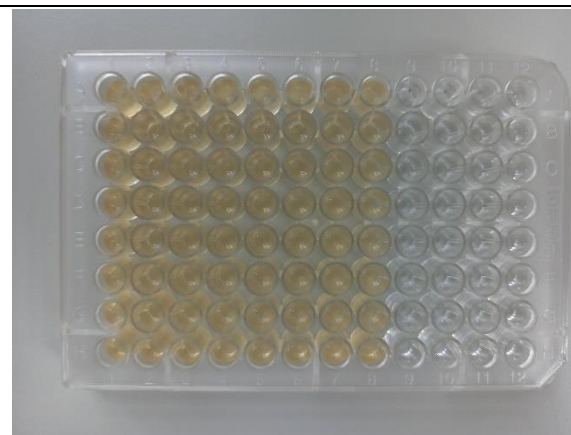
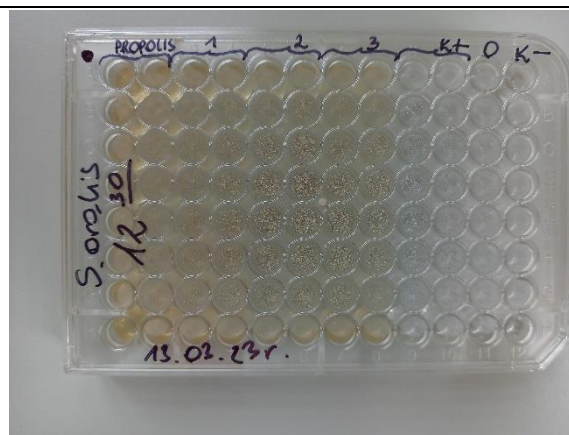
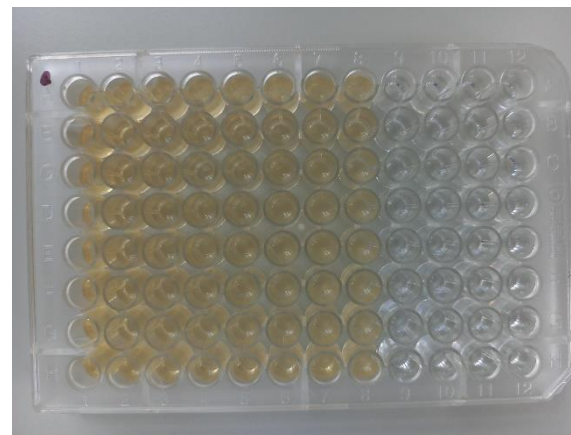
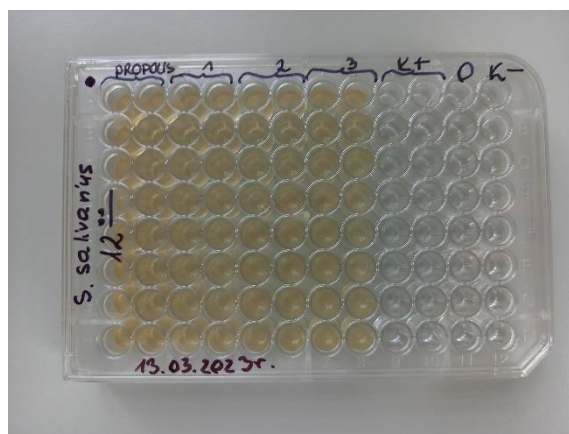
Table S7. Results of Fisher's LSD Test for *S. salivarius* MIC values of tested lozenges.

| LSD test; variable <i>S. salivarius</i> | | | | | | | | |
|--|---------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Probabilities for Post Hoc Tests Error: Between MS = .00583. df = 49.000 | | | | | | | | |
| Cell No. | Formulation | {1} 1.3731 | {2} 1.1195 | {3} 1.0253 | {4} 1.1885 | {5} .05262 | {6} .09975 | {7} .05237 |
| 1 | PT | | 0.000000 | 0.000000 | 0.000014 | 0.000000 | 0.000000 | 0.000000 |
| 2 | PT/HT | 0.000000 | | 0.017122 | 0.076924 | 0.000000 | 0.000000 | 0.000000 |
| 3 | PT/UV30m | 0.000000 | 0.017122 | | 0.000088 | 0.000000 | 0.000000 | 0.000000 |
| 4 | PT/UV60m | 0.000014 | 0.076924 | 0.000088 | | 0.000000 | 0.000000 | 0.000000 |
| 5 | Control (saliva & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | | 0.223073 | 0.994803 |
| 6 | Control (tablets WP & bacteria) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.223073 | | 0.220665 |
| 7 | Control (saliva) | 0.000000 | 0.000000 | 0.000000 | 0.000000 | 0.994803 | 0.220665 | |

Results marked in red are statistically significant at the level $p \leq 0.05$.

Table S8. Photos presenting 96-well plates and plates with broth made to determine the minimum bactericidal concentration for prepared lozenges with EEP.

| Bacteria | Photos of the 96-well microtiter plates used to determination of the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of EEP | |
|-----------------------------|---|--|
| <i>Streptococcus mitis</i> |  |  |
| <i>Streptococcus mutans</i> |  |  |

Streptococcus oralis*Streptococcus salivarius*

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.