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SYNERGISTIC ANTIBACTERIAL EFFECT OF PHENOLIC ACIDS AGAINST *ESCHERICHIA COLI*

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Summary. The aim of the study was to demonstrate the interactions occurring in the mixtures of phenolic acids and their effect on the growth of *Escherichia coli* ATCC 25922 and biofilm formation. The type of interaction between phenolic acids was determined using chessboard methods. The amount of the resulting biofilm was determined with spectrophotometric analysis. We found a synergy or partial synergy between phenolic acids in limiting the growth of *E. coli* bacteria. All the examined acids, i.e.: gallic (Gala), gentisic (Gena), vanillic (Va), *p*-coumaric (*p*Ka), and *trans*-cinnamic (*t*Ca), and their mixtures inhibited the growth of the *E. coli*. The most effective *E. coli* inhibitory effect was observed for a mixture of three acids: *t*Ca, *p*Ka, and Gena. All the considered acids and their mixtures significantly reduced the formation of biofilm by *E. coli*. Synergy or partial antibacterial synergy between examined phenolic acids against *E. coli* have been found.

Key words: synergy, antimicrobials, *Escherichia coli*, phenolic acids, biofilms

INTRODUCTION

Diseases caused by pathogenic bacteria transmitted by water and food still constitute a serious health problem in the world [Shan et al. 2007, EFSA and ECDC 2018]. In the European Union countries, 6.2% food poisonings are caused by *E. coli* producing a shiga toxin (STEC) [EFSA 2018].

One of the methods to prevent food contamination is the use of preservatives. Despite the effectiveness of these chemicals in controlling the growth of microorganisms, some people are afraid of the effects of their use in food [Shan et al. 2007, Hąc-Wydro et al. 2017]. Because of such concerns, efforts are being made to develop potentially effective, safer, and natural food preservatives. In this context, the use of plant extracts or their con-

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stituents as antimicrobials for food preservation appears to be a legitimate idea [Alzoreky and Nakahara 2003, Hara-Kudo et al. 2004, Khorshidian et al. 2018].

Many authors have reported that plant extracts owe their antimicrobial properties to ingredients such as phenolic compounds, tannins, catechins, and aldehydes [Ho et al. 2010, Medini et al. 2014, Prabakaran et al. 2018, Torres et al. 2018, Majeed et al. 2019]. Phenolic compounds are characterized by high biological activity. Because of this property, they are considered to be health-promoting food ingredients [Mattila and Hellström 2007, Heleno et al. 2015]. Among the phenolic compounds, a large group is represented by phenolic acids, which include hydroxycinnamic acid derivatives, i.e.: caffeic, *p*-coumaric, sinapinic, and ferulic, or hydroxybenzoic acid derivatives, i.e.: gallic, *p*-hydroxybenzoic, protocatechuic, and vanillic [Heleno et al. 2015].

Studies on single phenolic compounds, their mixtures, and full multicomponent extracts obtained from plants indicate that synergistic interactions can occur between them. It can be concluded based on previous studies that a natural combination of these compounds in extracts exhibits higher antimicrobial activity than single components [Dupont et al. 2006]. Interactions that can occur between individual compounds can be of a very different nature starting from synergistic interactions, additive effects, to antagonistic effects. In the future, the use of various combinations of already known antimicrobials, will develop [Davidson et al. 2013].

The aim of the study was to demonstrate the interactions occurring in the mixtures of phenolic acids and their effect on the growth of *E. coli* and influence on the biofilm formation.

MATERIAL AND METHODS

Biological material and phenolic acids

The study was conducted with *Escherichia coli* ATCC 25922. Phenolic acids, i.e.: gallic (Gala) (≥ 97.5%), *p*-coumaric (*p*Ka) (≥ 98%), *trans*-cinnamic (*t*Ca) (≥ 99%), vanillic (Va) (≥ 97%), and gentisic (Gena) (98%), were purchased from Sigma-Aldrich (St. Louis, USA). The basic solutions of acids were prepared in 96% v/v ethanol (Avantor, Poland) at a concentration of 10% w/v.

Determination of minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of phenolic acids, the serial microdilution method according to the National Committee for Clinical Laboratory Standards [2009a, b], was used. Two dilution series of the examined acids were prepared in Müller–Hinton broth (MHB, Merck, Germany) in the concentration range of 0.09–6.0 mg·mL^{-1} using 96-well plates. The inoculum of *E. coli* was added to each well containing 250 µL (MHB with the acids); concentration of $5 \cdot 10^5$ cfu·mL^{-1}. Inoculated plats were incubated at 37°C for 20 h. Resazurin (25 µL) at a concentration of 0.02% w/v (filtered through a sterile 0.22-µm filter) was added to each cell after 20 h than the plates were then incubated at 37°C for 2 h. The bacterial growth was evaluated based on the

changes in the resazurin color from purple to pink [Satyajit et al. 2007]. The MIC value was defined as the lowest concentration of phenolic acid in which no *E. coli* growth was observed (no changes in the resazurin color). A mixture containing only MHB and the inoculum but no acid, was used as a positive control.

Interactions between phenolic acids

To determine the interactions in the mixtures of phenolic acids, the MIC chessboard method was used [Gutiérrez-Fernández et al. 2013]. Dilutions of two or three phenolic acids were mixed in concentrations from 1 MIC to 1/64 MIC, MIC values of individual acids. *Escherichia coli* inoculum ($5 \cdot 10^5$ cfu·mL^{-1}) was added to each well in a plate containing 250 μL (MHB together with a mixture of acids). It was then continued as described above. The MIC value was then transformed into the fractional inhibitors concentration index (FICI). The FIC of the individual phenolic acid in the mixture was calculated according to the formula: $FIC_A = MIC_{AB} / MIC_A$ and $FIC_B = MIC_{AB} / MIC_B$, where MIC_{AB} is the minimum inhibitory concentration of a mixture of two acids, and MIC_A or MIC_B is the minimum inhibitory concentration of acid A or B. The FIC of individual components was calculated analogously for mixtures of three components: $FIC_A = MIC_{ABC} / MIC_A$, $FIC_B = MIC_{ABC} / MIC_B$, and $FIC_C = MIC_{ABC} / MIC_C$, where MIC_{ABC} is the minimum inhibitory concentration of a mixture of three acids and MIC_A, MIC_B, or MIC_C is the minimum inhibitory concentration of acid A, B, or C. The type of interaction that occurred in the examined mixtures was calculated as the sum of FIC for the individual components of the mixture in accordance with the formula: $FICI = FIC_A + FIC_B$ or $FICI = FIC_A + FIC_B + FIC_C$. The results were interpreted according to Liu et al. [2015]: total synergy $FICI \leq 0.5$, partial synergy $0.5 < FICI \leq 0.75$, no effect $0.75 < FICI \leq 2$, and antagonism $FICI > 2$.

Biofilm formation

Biofilm formation was investigated using the method described by Al-Shabib et al. [2017] with some modifications. Acids and their two- and three-component mixtures at concentrations ranging from MIC to 1/16 MIC were added to Luria–Bertani (LB) broth (Oxoid). The broth was then inoculated with *E. coli* to a final concentration of $5 \cdot 10^5$ cfu·mL^{-1}. Then, the mixture was transferred to wells in a 100-well flat-bottomed polystyrene plate (Honeycom, with a flat bottom) and incubated at 37°C for 48 h. The plates were then gently washed three times with phosphate buffered saline (PBS, pH 7.4) and stained with 100 mL of 0.1% w/v crystal violet (Sigma-Aldrich, USA) for 30 min at room temperature (25°C). The excess of crystal violet was removed by washing the plates three times with PBS; the plates were then resuspended in 150 μL of 96% v/v ethanol. The biofilm was quantified by measuring the optical density (OD) at the wavelength $\lambda = 600$ nm. The tests were performed in triplicate. The difference between the OD_{600} value of the LB medium and the sample containing phenolic acids or their mixtures was used to assess ability of bacteria to form biofilm formation. The classification was performed in accordance with the criteria set by Stepanovic et al. [2007]: samples with OD values between 0.1 and 0.4 had poor biofilm formation, and moderate biofilm formation was demonstrated at $OD_{600} > 0.4$ and strong biofilm formation at $OD_{600} > 0.8$.

Statistical analysis

Statistical analysis including the calculation of the arithmetic mean and the standard deviation was performed for all of the obtained results.

A normality test for OD biofilms was performed using the Shapiro–Wilk test and a homogeneity test of variance using the Levene or Brown–Forsythe tests. The significance of differences between the mean values was verified using an analysis of variance (ANOVA). Tukey's test was used to verify the differences between the mean values. All the calculations were conducted at a level of significance equal $p \leq 0.05$. All the statistical calculations were performed using Statistica 10 PL (StatSoft Poland) and Excel 2010 (Microsoft).

RESULTS AND DISCUSSION

All the examined phenolic acids inhibited the growth of *Escherichia coli* (Table 1). The highest inhibition was observed for Gala at the MIC of 0.375 mg·mL^{-1}, the MIC values of the other acids were 1.5 mg·mL^{-1}.

Table 1. Minimum inhibitory concentrations of phenolic acids against *E. coli*

Tabela 1. Minimalne stężenie kwasów fenolowych hamujące wzrost *E. coli*

Phenolic acid Kwas fenolowy	MIC [mg·mL^{-1}]
Gallic – Galusowy (Gala)	0.375
Gentisic – Gentyzynowy (Gena)	1.5
Vanillic – Wanilowy (Va)	1.5
p-Coumaric – *p*-Kumarynowy (*p*Ka)	1.5
trans-Cinnamic – *trans*-Cynamonowy (*t*Ca)	1.5

The inhibitory effects of the examined phenolic acids on *E. coli* has already been researched. Tuncel and Nergiz [1993] reported that the MIC value of *p*-coumaric acid was lower than the values in the present study (0.45 mg·mL^{-1}). Herald and Davidson [1983] found that *p*-coumaric acid at a concentration of 1 mg·mL^{-1} inhibited the growth of *E. coli*. Merkl et al. [2010] set the MIC value of gentisic acid to 0.45 mg·mL^{-1} in relation to *E. coli* DMF 7503. Carvalho et al. [2018] set the MIC value of gallic acid to 0.25 mg·mL^{-1} in relation to *E. coli*. According to Borges et al. [2012], the strong action of gallic acid is related to the higher hydroxylation of benzoic acid with respect to its other derivatives. Further, 0.5–2 mg of gallic acid in the disk-diffusion method showed weak or negligible activity against *E. coli* ATCC 25922 [Rauha et al. 2000, Vaquero et al. 2007a, b]. Merkl et al. [2010] found that the MIC for vanillic acid was 1.8 mg·mL^{-1} with respect to *E. coli*, which is the result at a level identical to that obtained in the present study. The discrepancy in the abovementioned results can be attributed mainly to different bacterial strains and the differences in the individual research methods.

The most frequently described mechanism of the action of phenolic compounds on microbial cells is the impairment or inhibition of the cell cytoplasmic membrane function.

Some chemical groups that build molecules of phenolic compounds, such as hydroxyl groups, have the ability to incorporate into the bacterial lipid membranes. The effect of this action is the reduction of the membrane potential, change in the permeability and fluidity of the membranes, and consequently cell lysis [Mirzoeva et al. 1997, Haraguchi et al. 1998, Cushine and Lamb 2005, Sirk et al. 2009].

Table 2 presents the types of interactions determined in two- and three-component mixtures.

Table 2. Antimicrobial activity of mixtures of phenolic acids against *E. coli*

Tabela 2. Działanie przeciwdrobnoustrojowe mieszanin kwasów fenolowych na *E. coli*

Mixture – Mieszanina			FIC_A	FIC_B	FIC_C	FICI	Type of interaction Typ interakcji
acid kwas A	acid kwas B	acid kwas C					
*t*Ca	*p*Ka	×	0.50	0.02	×	0.52	PS
*t*Ca	Gala	×	0.50	0.06	×	0.56	PS
Va	*p*Ka	×	0.50	0.06	×	0.56	PS
*t*Ca	Gena	×	0.50	0.13	×	0.63	PS
*t*Ca	Va	×	0.50	0.13	×	0.63	PS
Gena	*p*Ka	×	0.50	0.25	×	0.75	PS
Va	Gena	×	0.50	0.25	×	0.75	PS
Va	Gala		0.50	0.50		1.00	NE
*p*Ka	Gala	×	0.50	0.50	×	1.00	NE
Gena	Gala	×	0.50	0.50	×	1.00	NE
Va	*t*Ca	Gena	0.25	0.13	0.13	0.50	S
*t*Ca	*p*Ka	Gena	0.25	0.13	0.13	0.50	S
*t*Ca	*p*Ka	Va	0.50	0.02	0.02	0.54	PS
Gala	*t*Ca	Gena	0.50	0.02	0.02	0.54	PS
Va	*t*Ca	Gala	0.25	0.25	0.06	0.56	PS
Va	*p*Ka	Gala	0.50	0.02	0.06	0.58	PS
Va	Gena	*p*Ka	0.50	0.13	0.06	0.68	PS
*p*Ka	Gala	*t*Ca	0.50	0.13	0.06	0.68	PS
Va	Gena	Gala	0.50	0.13	0.06	0.68	PS
Gena	*p*Ka	Gala	0.50	0.25	0.06	0.81	NE

S – synergism; PS – partial synergism; NE – non effect.

S – synergia; PS – częściowa synergia; NE – brak efektu.

So far studies in this area have mainly focused on the interactions between various compound of essential oils or their components and other compounds belonging to phenols. Meira et al. [2017] showed the potential value of phenolic acids with compounds of

essential oils *in vitro* as an antimicrobial properties of against *E. coli* O157:H7. They presented synergism between *o*-coumaric acid and allyl isothiocyanate (FIC = 0.25). A FIC of 0.5 was shown by three mixtures, the first one was carvacrol and *o*-coumaric acid, the second allyl isothiocyanate and *p*-hydroxybenzoic acids and the third one allyl isothiocyanate and ferulic acid. Oliveira et al. [2019] demonstrated a processing approach based on synergistic antimicrobial activity of two phenolic acids (gallic acid and ferulic acid) which in combination with mild levels of physical stresses in the form of light, heat, or pressure were able to lower *E. coli* O157:H7 in clarified apple juice. Some of the observed synergistic combinations, such as ferulic acids and light (UV-A) or gallic acid with mild heat (55°C) or a moderate pressure (250 MPa) caused inactivation of *E. coli* O157:H7 in apple juice by more than 4 log cfu·mL^{-1}.

For instance a synergistic effect of the thymol–carvacrol mixture with respect to the *Enterococcus faecalis* strains was found. Partial synergy against *E. faecalis* was also demonstrated for carvacrol and gallic acid. Thymol in combination with gallic acid was found to show a partial synergy or additive effect depending on the strain (FIC = 0.88–1.00) [Gutiérrez-Fernández et al. 2013]. Synergy was also demonstrated for polyphenols in combination with fatty acids. Caprylic acid and thymol used separately against the pathogenic strain of *E. coli* O157:H7 caused a reduction in the number of bacterial cells by 0.2 log, while their combination resulted in a decrease in the number of cells by 7.47 logs. The same results were obtained by combining caprylic acid and carvone. The mixtures of these fatty acids, decanoic acid, and lauric acid with thymol, carvone, and eugenol or vanillin showed a slightly lower effect [Kim and Rhee 2015].

In our study we have found, that phenolic acids and their mixtures affected the ability of biofilm formation by *E. coli* (Table 3).

The OD_{600} value of 0.52 for the *E. coli* ATCC 25922 biofilm in the control medium indicated that this strain belonged to the category of moderate biofilm formants [Stepanovic et al. 2007]. In the medium containing sub-MIC concentrations of acids, *E. coli* cells proliferated to values close to the control group (LB + *E. coli*, data not shown). All the examined phenolic acids inhibited the *E. coli* biofilm production, and the biofilm reduction ranged from 71 to 97% compared to the control sample. The OD_{600} values for all the concentrations of single phenolic acids did not exceed the value of 0.1, which indicated that *E. coli* could not produce biofilms. The combination of two acids led to better results at lower doses, which indicated a synergistic effect of the acid mixtures on the inhibition of biofilm formation by *E. coli*. The mixture of Va and *p*Ka inhibited biofilm production by *E. coli* more effectively than when these acids were used separately. Two-component mixtures containing *t*Ca showed greater inhibition of biofilm formation by *E. coli* than when the acids were used separately. The highest efficiency in biofilm reduction was demonstrated by *t*Ca and Gala mixtures, followed by the mixtures of *t*Ca and Gena as well as *t*Ca and Va. Three-component mixtures limited the biofilm formation even further. The most effective mixtures were as follows: *p*Ka, Gala, and *t*Ca as well as Va, Gena, and Gala. Weak biofilm formation by *E. coli* was observed in the presence of mixtures (*t*Ca, *p*Ka, and Gena, *t*Ca, *p*Ka, and Va; Gala, *t*Ca, and Gena; and Va, *t*Ca, and Gala). Statistical analysis of the results indicated significant difference ($p \leq 0.05$) between the control medium, and the medium containing phenolic acids, where no biofilm formation by *E. coli* was observed. We found that in spite of the decreasing concentration of acids, biofilm

Table 3. Impact of phenolic acids or their mixtures on *E. coli* ability to form biofilms

Tabela 3. Wpływ kwasów fenolowych oraz ich mieszanin na zdolność tworzenia biofilmów przez *E. coli*

Mixture – Mieszanina			$\Delta OD_{600} \pm SD$				
acid kwas A	acid kwas B	acid kwas C	MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
Gala		×	0.045 ±0.001^{aA}	0.049 ±0.008^{aA}	0.096 ±0.033^{bA}	0.028 ±0.007^{aA}	0.027 ±0.010^{aA}
Gena		×	0.037 ±0.003^{aA}	0.052 ±0.019^{aA}	0.053 ±0.009^{aA}	0.080 ±0.014^{aA}	0.075 ±0.030^{aA}
*t*Ca		×	0.028 ±0.002^{aA}	0.027 ±0.001^{aA}	0.038 ±0.015^{aA}	0.049 ±0.018^{aA}	0.052 ±0.005^{aA}
*p*Ka		×	0.031 ±0.010^{aA}	0.030 ±0.011^{aA}	0.031 ±0.012^{aA}	0.042 ±0.034^{aA}	0.094 ±0.022^{bA}
Va		×	0.047 ±0.020^{abA}	0.061 ±0.045^{abA}	0.036 ±0.009^{aA}	0.052 ±0.004^{aA}	0.045 ±0.021^{aA}
*t*Ca	*p*Ka	×	0.018 ±0.022^{aA}	0.009 ±0.002^{aA}	0.019 ±0.004^{aA}	0.037 ±0.027^{aA}	0.011 ±0.007^{aA}
*t*Ca	Gala	×	0.040 ±0.017^{bA}	0.009 ±0.007^{aA}	0.011 ±0.001^{aA}	0.022 ±0.005^{abA}	0.023 ±0.006^{abA}
Va	*p*Ka	×	0.023 ±0.014^{aA}	0.036 ±0.014^{aA}	0.024 ±0.009^{aA}	0.013 ±0.012^{aA}	0.044 ±0.019^{aA}
*t*Ca	Gena	×	0.019 ±0.025^{aA}	0.011 ±0.010^{aA}	0.007 ±0.009^{aA}	0.027 ±0.013^{aA}	0.026 ±0.009^{aA}
*t*Ca	Va	×	0.014 ±0.005^{abA}	0.013 ±0.010^{abA}	0.028 ±0.015^{bA}	0.032 ±0.005^{bA}	0.024 ±0.004^{aA}
*p*Ka	Gena	×	0.014 ±0.012^{aA}	0.026 ±0.010^{aA}	0.048 ±0.014^{bA}	0.039 ±0.015^{abA}	0.035 ±0.013^{abA}
Va	Gena	×	0.008 ±0.004^{aA}	0.020 ±0.007^{aA}	0.002 ±0.001^{aA}	0.020 ±0.020^{aA}	0.025 ±0.013^{aA}
Va	Gala	×	0.035 ±0.011^{aA}	0.028 ±0.013^{aA}	0.014 ±0.017^{aA}	0.053 ±0.011^{bA}	0.042 ±0.013^{bA}
*p*Ka	Gala	×	0.024 ±0.011^{aA}	0.025 ±0.020^{aA}	0.037 ±0.017^{aA}	0.033 ±0.011^{aA}	0.026 ±0.013^{aA}
Gena	Gala	×	0.046 ±0.012^{cA}	0.037 ±0.005^{bcA}	0.020 ±0.002^{abA}	0.025 ±0.014^{ab}	0.020 ±0.007^{abA}

Table 3. cont.

Tabela 3. cd.

Mixture – Mieszanina			$\Delta OD_{600} \pm SD$				
acid kwas A	acid kwas B	acid kwas C	MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
Va	*t*Ca	Gena	0.020 ±0.006^{aA}	0.039 ±0.027^{aA}	0.066 ±0.029^{aA}	0.064 ±0.020^{aA}	0.123 ±0.042^{aA}
*t*Ca	*p*Ka	Gena	0.108 ±0.006^{aA}	0.081 ±0.045^{aA}	0.084 ±0.028^{aA}	0.108 ±0.024^{aA}	0.080 ±0.005^{aA}
*t*Ca	*p*Ka	Va	0.050 ±0.030^{aA}	0.139 ±0.018^{bA}	0.133 ±0.030^{bA}	0.118 ±0.018^{bA}	0.138 ±0.026^{bA}
Gala	*t*Ca	Gena	0.105 ±0.022^{aA}	0.094 ±0.007^{aA}	0.100 ±0.036^{aA}	0.083 ±0.009^{aA}	0.126 ±0.015^{aA}
Va	*t*Ca	Gala	0.087 ±0.013^{aA}	0.114 ±0.007^{aA}	0.129 ±0.006^{aA}	0.102 ±0.005^{aA}	0.095 ±0.035^{aA}
Va	*p*Ka	Gala	0.056 ±0.024^{aA}	0.068 ±0.016^{aA}	0.092 ±0.008^{aA}	0.099 ±0.038^{aA}	0.085 ±0.018^{aA}
Va	Gena	*p*Ka	0.096 ±0.049^{bA}	0.045 ±0.015^{abA}	0.043 ±0.009^{abA}	0.038 ±0.011^{abA}	0.026 ±0.010^{aA}
*p*Ka	Gala	*t*Ca	0.016 ±0.002^{aA}	0.011 ±0.004^{aA}	0.009 ±0.001^{aA}	0.013 ±0.003^{aA}	0.009 ±0.004^{aA}
Va	Gena	Gala	0.009 ±0.002^{abA}	0.006 ±0.002^{aA}	0.016 ±0.002^{cA}	0.011 ±0.00^{abcA}	0.014 ±0.004^{bcA}
Gena	*p*Ka	Gala	0.005 ±0.003^{aA}	0.017 ±0.004^{bA}	0.014 ±0.003^{abA}	0.012 ±0.003^{abA}	0.018 ±0.002^{bA}
Control – Kontrola			0.520 ±0.065^{B}	×	×	×	×

^{abc} Homogeneous groups of effect of phenolic acids or mixtures of phenolic acids on ability to form biofilms (a separate analysis was conducted for each acids concentration, in rows) – Jednorodne grupy wpływu kwasów fenolowych lub mieszanin kwasów fenolowych na zdolność do tworzenia biofilmów (przeprowadzono odrębną analizę dla każdego stężenia kwasów, w wierszach).

^{AB} Homogeneous groups of effect of phenolic acids or mixtures of phenolic acids on the ability to form biofilms with respect to the control – Jednorodne grupy wpływu kwasów fenolowych lub mieszanin kwasów fenolowych na zdolność do tworzenia biofilmów w stosunku do kontroli.

formation was inhibited to the same extent, as evidenced by the absence of significant differences ($p \leq 0.05$).

Borges et al. [2012] investigated the effect of two phenolic acids, i.e.: gallic and ferulic, on preventing biofilm formation by *E. coli*. These phenolic acids acted preventively on the formation of biofilms and caused the reduction of the biofilm mass. Ugurlu et al. [2016] examined the effect of phenolic acids, i.e.: vanillic, caffeic, cinnamic, and ferulic, on the intercellular communication and mobility of *Pseudomonas aeruginosa* cells. The addition of cinnamic, ferulic or vanillic acid in to the medium reduced the production of biofilms as compared to the control sample. These acids inhibited the secretion of signaling molecules during *quorum sensing*, which is necessary for biofilm formation. The application of phenolic acids reduced the biofilm formation by approximately 50% [Borges et al. 2012]. Cui et al. [2020] proved the reduction of *E. coli* biofilm by clove oil. Some of the surface of the bacteria collapsed, which may be caused by the exudation of the contents.

CONCLUSIONS

In this study, we found a synergy or partial synergy between phenolic acids for limiting the growth of the *E. coli*. All the examined acids, i.e.: Gala, *p*Ka, Gena, *t*Ca, and Va, and their mixtures inhibited the growth of the *E. coli* ATCC 25922. The most effective *E. coli* ATCC 25922 inhibitory effect was observed for a mixture of three acids: *t*Ca, *p*Ka, and Gena, which also in the shortest time led to the killing of the bacterial cells. All the considered acids and their mixtures significantly reduced the biofilm formation by *E. coli*.

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SYNERGISTYCZNE DZIAŁANIE PRZECIWBAKTERYJNE KWASÓW FENOLOWYCH PRZECIWKO *ESCHERICHIA COLI*

Streszczenie. Celem badań było wykazanie interakcji zachodzących w mieszaninach kwasów fenolowych oraz określenie ich wpływu na wzrost *Escherichia coli* ATCC 25922 i zdolność tworzenia biofilmu. Rodzaj interakcji między kwasami fenolowymi oznaczono

metodą szachownicy. Ilość powstałego biofilmu oznaczono spektrofotometrycznie. Stwierdzono występowanie zjawiska synergii lub częściowej synergii między kwasami fenolowymi w ograniczeniu wzrostu bakterii *E. coli*. Wszystkie badane kwasy, tj.: galusowy (Gala), gentyzynowy (Gena), wanilowy (Va), *p*-kumarynowy (*p*Ka) i *trans*-cynamonowy (*t*Ca), oraz ich mieszaniny hamowały wzrost *E. coli*. Największy efekt hamujący wzrost *E. coli* zaobserwowano dla mieszaniny trzech kwasów: *t*Ca, *p*Ka i Gena. Wszystkie badane kwasy i ich mieszaniny znacznie zmniejszyły ilość tworzonego biofilmu przez *E. coli*.

Słowa kluczowe: synergia, środki przeciwdrobnoustrojowe, *Escherichia coli*, kwasy fenolowe, biofilmy