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Evaluation of the antimicrobial and antibiofilm activity of sweet cherry (*Prunus avium* L.) stems extracts

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Abstract

Sweet cherry (*Prunus avium* L.) stems in the form of infusions and decoctions are traditionally consumed for diuretic and anti-inflammatory purposes. This study aimed to evaluate antimicrobial and antibiofilm activity of ethanolic and methanolic extract made from sweet cherry stems. Extracts are obtained by the Soxhlet extraction and maceration procedures. For the determination of the minimum inhibitory concentration, the broth microdilution method is employed, and the assessment of the microbiocidal activity of the extracts is conducted. The antibiofilm activity was tested through the tissue culture plate method, which also allowed the determination of the biofilm-forming categories of investigated strains. The final step involved the calculation of the biofilm inhibition percentage. Examined extracts with the balanced activity inhibited the growth of all microorganisms, with Gram-negative bacteria being more sensitive in comparison to Gram-positive. The values of the minimum inhibitory concentration were 125 µg/ml, and 250 µg/ml, respectfully. *Candida albicans* was the most susceptible and the minimum inhibitory concentration of both extracts was 62.50 µg/ml. The microbiocidal activity of the extracts was not recorded. Extracts exhibited different impacts on the biofilm-forming capacity of the investigated microbes, and both inhibition and stimulation effects are noted. The percentage of the biofilm inhibition was from 14.27% to 84.78%, with the highest inhibition recorded for the multidrug-resistant *Escherichia coli*, treated with the ethanolic extract. Sweet cherry stems are a valuable source of natural bioactive compounds, but their usage in the treatment of microbial infections should be correctly and carefully implemented.

Introduction

Prunus avium L., known as sweet cherry or wild cherry is a deciduous tree belonging to the Rosaceae family, that is widespread throughout the world, especially in temperate climate regions. Traditional medicine recognizes the beneficial effects of this plant for centuries, and sweet cherry stems are often used in form of infusions and decoctions due to their different bioactive properties (Afonso et al., 2020). The therapeutic potential of the sweet cherry stems is mainly associated with the urinary tract, where different products are used as diuretic, draining, and anti-inflammatory agents (Nunes et al., 2021).

In Bosnia and Herzegovina, tea preparations from sweet cherry stems are consumed in folk medicine for centuries, mostly for diuretic purposes and for the promotion of proper kidney function (Ademović et al., 2017). Previous studies regarding the antibacterial and antibiofilm activity of various sweet cherry products (Rovčanin et al., 2015; Ademović et al., 2017; Oyetayo and Bada, 2017; Abedini et al., 2020; Afonso et al., 2020; Nunes et al., 2021; Ortega-Vidal et al., 2021) showed that this plant species have promising antimicrobial potential.

Today, it is known that increased antimicrobial resistance induced by the overuse of antibiotics represents a global public health challenge (Llor and Bjerrum, 2014). Issues associated with biofilm occurrence, as the predominant mode of microbial growth, and also one of the key survival strategies in which microbes can resist a number of chemical and physical stresses (Altaf et al., 2021), led to the growing demand for new antimicrobial agents, especially those of natural origin (Vaou et al., 2021). The main goal of this investigation was to evaluate the antimicrobial potential of ethanolic and methanolic extract made from sweet cherry stems, as well as to assess the impact of these extracts on the biofilm-forming capacity of different bacterial and fungal species.

Material and methods

Plant material

Sweet cherry stems were collected from a healthy adult sweet cherry tree (*Prunus avium* L.) located in the Mostar area (Bosnia and Herzegovina) in August 2019. Biological authentication was carried out in Laboratory for Plant Systematics, Department of Biology, University of Sarajevo-Faculty of Science. Upon washing, the material was dried in shade for 30 days. Dry stems were homogenized and stored at 4 °C until further analyses.

Extraction procedures

Ethanolic and methanolic extract of sweet cherry stems were made by the Soxhlet extraction and maceration extraction method respectfully, by using the amount of 80 ml of solvent (Sigma Aldrich) and 4 g of sample. The extracts were filtered, concentrated on a rotating vacuum evaporator, and stored in a dark place at 4-6 °C. For microbiological investigation extracts were dissolved in dimethyl sulfoxide (DMSO; Sigma Aldrich) to the final concentration of 1000 μg/ml. All reagents were of high analytical grade.

Microorganisms

Antimicrobial and antibiofilm activity of extracts was tested against 11 Gram-positive and Gram-negative bacteria and one fungus, including the multidrug-resistant (MDR) strains: *Staphylococcus aureus* ATCC 6538 (SA1); *S. aureus* ATCC 25923 (SA2); methicillin-resistant *S. aureus* (MRSA): *S. aureus* ATCC 33591 (SA3), and *S. aureus* NCTC 12493 (SA4); *Enterococcus faecalis* ATCC 29212 (EF); *Bacillus subtilis* ATCC 6633 (BS); *Escherichia coli* ATCC 14169 (EC1); *E. coli* ATCC 25922 (EC2); extended-spectrum beta-lactamase-producing (ESBL) *E. coli* ATCC 35218 (EC3); *Salmonella enterica* NCTC 6017 (SE);

Pseudomonas aeruginosa ATCC 27853 (PA), and *Candida albicans* ATCC 10231. In order to obtain inoculums, bacterial strains were at first cultured overnight at 37 °C in Mueller Hinton (MH) medium (Sigma Aldrich), and fungal strain in Sabouraud Glucose Agar (SGA) (Sigma Aldrich), and then dissolved in sterile saline solution corresponding to the 0.5 McFarland standard and microbial cell concentration of 1.5×10^8 CFU/ml. Inoculums were prepared according to the recommendation of (EUCAST, 2017).

Determination of the minimum inhibitory concentration and minimum microbiocidal concentration

The broth microdilution method (CLSI, 2018) was used for the determination of minimum inhibitory concentration (MIC) of investigated extracts.
The volume of 100 µl of two-fold dilutions of extracts in the range of 1000-1.95 µg/ml was added to a 96-well microtiter plate containing the Mueller Hinton Broth (Sigma Aldrich). For *C. albicans* Sabouraud Glucose Broth (Sigma-Aldrich) was used. After that, 10 µl of microbial inoculum was added to each well. The pure microbial culture was taken as the positive control, while the negative control was the uninoculated media. After overnight incubation, results were read on a microplate reader (Biochrom EZ Read 400) at 595 nm. All experiments were performed in quadruplets. In order to evaluate the minimum bactericidal (MBC) and minimum fungicidal concentration (MFC) of the extracts, bacterial and fungal strains were replated on sterile MH medium and SGA respectfully, and after overnight incubation, the presence of the microbial growth is observed. These experiments were done in triplicate.

Evaluation of antibiofilm activity

Antibiofilm activity of sweet cherry stems extracts was evaluated through the tissue culture plate method (TCP) in 96 well plates (Merritt et al., 2005). As the dilution medium, Tryptic Soy Broth (TSB, Sigma Aldrich) was used, and the initial concentration of extracts (1000 µg/ml) was two-fold diluted in TSB up to the end concentration of 1.95 µg/ml. The amount of 100 µl of such dilutions was added to the microtiter plate wells, followed by the inoculation with 10 µl of the investigated microbial strain. Inoculums were set as described earlier. As the negative control, uninoculated media was used.
The adherence of microbial strains only in the presence of TSB was used for the determination of biofilm formation. After overnight incubation, the content of the plates was decanted, and plates were washed in Phosphate Buffered Saline (PBS, Sigma Aldrich), and stained for 10 minutes with 0.1% crystal violet solution. Upon washing, each well is filled with 96% ethanol, and the results were read on the microplate reader (Biochrom EZ Read 400) at 595 nm. The protocol was performed in four replications, and the results are given as the mean value ± STDEV. Biofilm-forming category was determined according to Stepanović et al. (2007), and by using the Biofilm Classifier Software ver 1.1. The optical density cut-off value (ODc) was calculated as three standard deviations above the mean OD of the negative control, while the biofilm-forming categories were determined as follows: OD ≤ ODc: non-adherent (NA), ODc < OD ≤ 2 x ODc: weakly adherent (W), 2 x ODc < OD ≤ 4 x ODc: moderately adherent (M), and 4 x ODc < OD: strongly adherent (S). The percentage of biofilm inhibition achieved by the extract activity was calculated according to Jadhav et al. (2013).

Results and Discussion

Inhibitory activity

All included microorganisms were successfully inhibited by ethanolic and methanolic extract of sweet cherry stems. Results of MIC determination showed that tested Gram-negative bacteria are

more susceptible (MIC=125 μg/ml) to investigated extracts in comparison to Gram-positive strains (MIC=250 μg/ml), while *C. albicans* was the most sensitive strain with MIC=62.50 μg/ml. Ethanolic and methanolic extract performed balanced activity (Table 1).

After replating the microorganisms in order to determine the minimum microbiocidal concentrations, microbial growth was observed, which suggested that investigated extracts don't perform the microbiocidal activity. Results gained with the investigation of the impact of the extracts on the biofilm formation provide more insights into the antimicrobial potential of sweet cherry stems extracts.

Determination of the biofilm-forming categories

Results regarding the biofilm-forming capacity alteration in presence of different concentrations of extract compared to the positive control, where no extract was added, were tested at the MIC value and at subinhibitory concentrations. The positive controls which were classified as strong biofilm formers included: SA1, SA2, SA4 (MRSA), EC3 (ESBL), and PA. SA3 (MRSA), EF, BS, EC1, and SE expressed weak biofilm-forming capacity, while EC2 used in this study did not show any biofilm-forming potential since all performed replications suggested the non-adherent biofilm category for this strain. CA was classified as a moderate biofilm-former. Results are presented in Figure 1.

Table 1. The minimum inhibitory concentration of tested sweet cherry stems extracts

Microbial strain	**MIC (μg/ml)**	
	Ethanolic extract	**Methanolic extract**
Staphylococcus aureus ATCC 6538	250	250
S. aureus ATCC 25923	250	250
S. aureus ATCC 33591	250	250
S. aureus NCTC 12493	250	250
Enterococcus faecalis ATCC 29212	250	250
Bacillus subtilis ATCC 6633	250	250
Escherichia coli ATCC 14169	125	125
E. coli ATCC 25922	125	125
E. coli ATCC 35218	125	125
Salmonella enterica NCTC 6017	125	125
Pseudomonas aeruginosa ATCC 27853	125	125
Candida albicans ATCC 10231	62.5	62.5

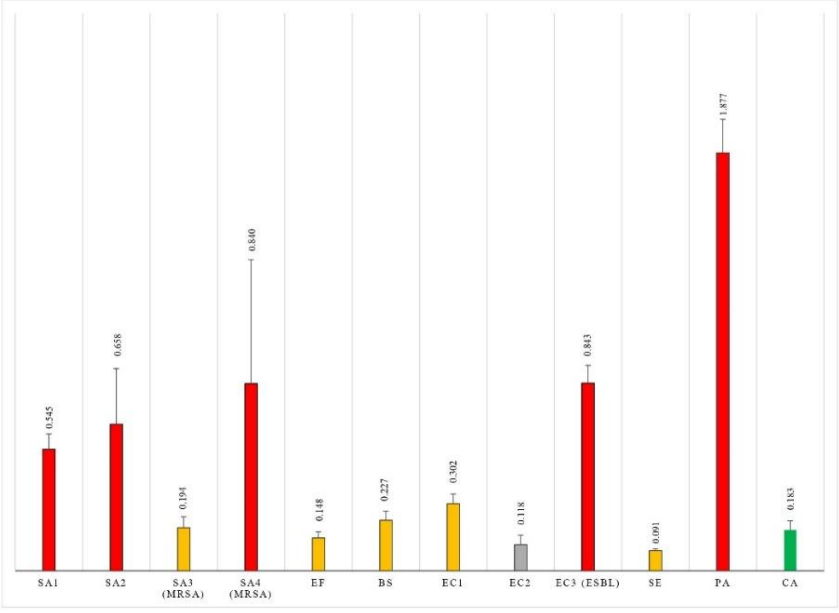

Figure 1. Determined categories of the biofilm-forming capacity of investigated microorganisms. SA1: *Staphylococcus aureus* ATCC 6538; SA2: *S. aureus* ATCC 25923; SA3: *S. aureus* ATCC 33591 (MRSA); SA4: *S. aureus* NCTC 12493 (MRSA); EF: *Enterococcus faecalis* ATCC 29212; BS: *Bacillus subtilis* ATCC 6633; EC1: *Escherichia coli* ATCC 14169; EC2: *E. coli* ATCC 25922; EC3: *E. coli* ATCC 35218 (ESBL); SE: *Salmonella enterica* NCTC 6017; PA: *Pseudomonas aeruginosa* ATCC 27853; CA: *Candida albicans* ATCC 10231.

Effects of the sweet cherry extracts on microbial biofilms

Ethanolic and methanolic extract of sweet cherry stems caused the decreasing of the biofilm-forming category at particular dilutions in two tested Gram-positive bacteria as follows. Ethanolic extract led to the forming of moderately adherent biofilm of SA2 in the range of 31.25-1.95 µg/ml, while methanolic extract prevented the biofilm formation of SA3 (MRSA) at concentrations of 125-31.25 µg/ml (Table 2). Strong biofilm formers SA1 and SA4 (MRSA) were resistant to the activity of examined extracts and there was no change in the biofilm-forming category. Interesting results are noticed in the case of EF and BS, classified as the weak biofilm-formers, where the application of the extracts mainly led to the increase of the biofilm-forming capacity. EF formed moderately adherent biofilm when treated with ethanolic extract in all subinhibitory doses, while methanolic extract even led to the formation of strong biofilm at particular dilutions (125-15.63 µg/ml).

Overall results suggest that Gram-negative bacteria were more susceptible to the examined extracts in terms of antibiofilm activity.

Specific concentrations of the ethanolic and methanolic extract successfully inhibited the biofilm formation of three Gram-negative bacteria: EC1, EC3 (ESBL), and PA. EC1, classified as the weak biofilm former, was more sensitive to the methanolic extract, where the first subinhibitory concentration disabled the biofilm formation. At

the same time, ethanolic extract led to an increase in the biofilm-forming capacity, and moderately- and strongly adherent biofilms were observed. EC3 (ESBL) biofilm was decreased to weakly- and moderately adherent at 62.50 µg/ml of ethanolic and methanolic extract respectfully. Strong PA biofilm was inhibited to moderately adherent by all subinhibitory concentrations of methanolic extract. There was no change in the biofilm-forming category in the case of SE. Furthermore, EC2 strain did not form the biofilm in all replications of the positive control, but after the application of the investigated extracts, this bacterial strain formed weakly- and even moderately adherent biofilms (Table 3).

Antibiofilm activity of the sweet cherry stems extracts against *C. albicans* was in accordance with the attained MIC values in this study. All subinhibitory concentrations of extracts led to the decrease in a biofilm-forming category from moderately- to weakly adherent (Table 4).

The percentage of the biofilm inhibition was calculated as previously mentioned and summarized in Figure 2. In comparison to the positive control, the highest biofilm inhibition was achieved in the case of the ethanolic extract against EC3 (ESBL), 84.78%. The methanolic extract inhibited the EC1 biofilm in the amount of 62.12%, and EC3 (ESBL) biofilm in the amount of 57.52%. Intermediate biofilm inhibition below 50% was achieved with methanolic extract against PA (33.52%) and SA3 (MRSA) (19.94%), as well as with ethanolic extract against SA2 (23.04%). CA biofilm was inhibited by both extracts, 26.29% with ethanolic and 14.27% with methanolic extract (Figure 2).

In terms of potential medicinal use of sweet cherry stems products, and due to the toxicity of methanol as a solvent, obtained results go in favor of ethanolic extracts since there was no difference in their antimicrobial activity. Earlier study regarding

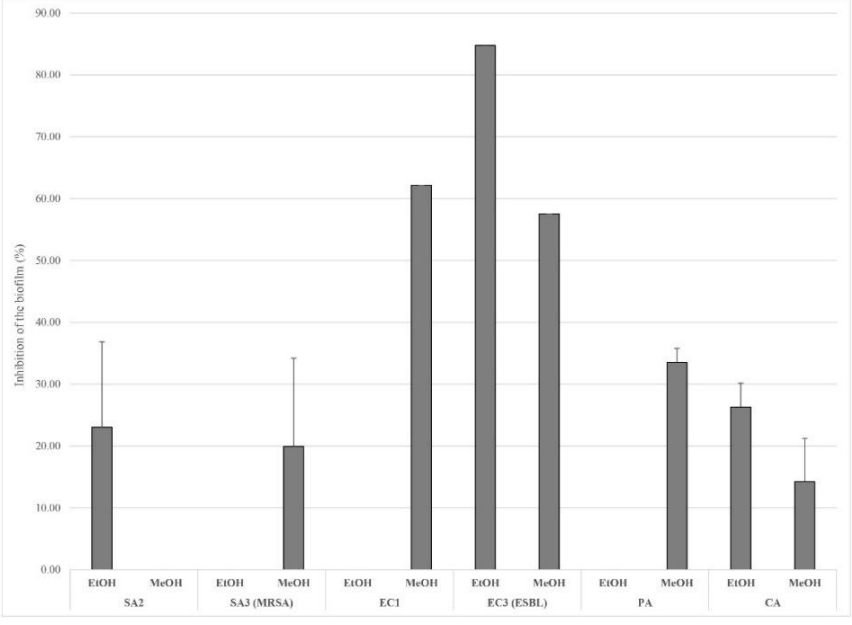

Figure 2. Percentage of the biofilm inhibition of tested microbial strains. SA2: *S. aureus* ATCC 25923; SA3: *S. aureus* ATCC 33591 (MRSA); EC1: *Escherichia coli* ATCC 14169; EC3: *E. coli* ATCC 35218 (ESBL); PA: *Pseudomonas aeruginosa* ATCC 27853 (PA); CA: *Candida albicans* ATCC 10231.

Table 2. Obtained TCP values after application of subinhibitory concentrations of extracts on Gram-positive bacteria

Bacterial strain	Solvent	Subinhibitory concentrations of extracts (µg/ml)						
		125	62.50	31.25	15.63	7.81	3.90	1.95
SA1	E	0.523±0.086	0.598±0.221	0.609±0.079	0.506±0.129	0.553±0.174	0.736±0.087	0.356±0.068
	M	0.529±0.118	0.675±0.077	0.798±0.139	0.583±0.122	0.736±0.194	0.747±0.070	0.508±0.130
SA2	E	0.823±0.049	0.805±0.171	0.644±0.134*	0.460±0.084*	0.468±0.148*	0.642±0.115*	0.670±0.065*
	M	O/R	O/R	O/R	2.430±1.046	0.753±0.314	1.626±0.913	1.569±1.093
SA3 (MRSA)	E	0.122±0.019	0.147±0.041	0.137±0.014	0.131±0.017	0.131±0.007	0.150±0.050	0.139±0.005
	M	0.152±0.026*	0.214±0.031*	0.206±0.032*	0.293±0.041	0.327±0.094	0.296±0.139	0.243±0.146
SA 4 (MRSA)	E	O/R	O/R	O/R	O/R	O/R	O/R	O/R
	M	O/R	O/R	O/R	O/R	O/R	O/R	O/R
EF	E	0.280±0.078**	0.280±0.056**	0.273±0.014**	0.240±0.030**	0.165±0.018**	0.161±0.012**	0.162±0.009**
	M	0.895±0.052**	0.766±0.208**	0.606±0.241**	0.446±0.144**	0.320±0.113**	0.220±0.064**	0.169±0.016
BS	E	0.286±0.065**	0.258±0.052**	0.274±0.083**	0.184±0.031**	0.166±0.012**	0.186±0.028**	0.240±0.036**
	M	0.598±0.287**	0.664±0.120**	0.603±0.133**	0.365±0.060	0.274±0.021	0.259±0.028	0.229±0.052

SA1: *Staphylococcus aureus* ATCC 6538; SA2: *S. aureus* ATCC 25923; SA3: *S. aureus* ATCC 33591 (MRSA); SA4: *S. aureus* NCTC 12493 (MRSA); EF: *Enterococcus faecalis* ATCC 29212; BS: *Bacillus subtilis* ATCC 6633.

E: ethanol; M: methanol.

O/R: Over the highest observed absorbance value.

Marked concentrations of extracts caused the change in the biofilm-forming capacity in comparison to the positive control.

*Decreasing of the biofilm-forming category or biofilm elimination.

**Increasing of the biofilm-forming category.

Table 3. Obtained TCP values after application of subinhibitory concentrations of extracts on Gram-negative bacteria

Bacterial strain	Solvent	Subinhibitory concentrations of extracts (µg/ml)					
		62.50	31.25	15.63	7.81	3.90	1.95
EC1	E	0.128±0.021	0.241±0.026**	0.389±0.025**	0.446±0.084**	0.447±0.035**	0.416±0.050**
	M	0.191±0.019*	0.333±0.054	0.465±0.055	0.473±0.051	0.484±0.019	0.573±0.157
EC2	E	0.271±0.018**	0.270±0.039**	0.172±0.022**	0.110±0.010**	0.081±0.008**	0.078±0.005**
	M	0.660±0.039**	1.107±0.217**	0.963±0.191**	0.628±0.132**	0.348±0.110	0.205±0.046
EC3 (ESBL)	E	0.126±0.039*	0.566±0.109	0.853±0.110	0.882±0.050	0.871±0.065	0.837±0.080
	M	0.364±0.036*	0.753±0.169	0.971±0.170	1.135±0.179	1.162±0.260	1.146±0.261
SE	E	0.121±0.024	0.122±0.008	0.113±0.005	0.120±0.013	0.101±0.035	0.101±0.013
	M	0.137±0.038	0.170±0.029	0.159±0.026	0.131±0.025	0.137±0.022	0.100±0.011
PA	E	0.425±0.092	0.948±0.184	1.255±0.363	1.030±0.104	0.980±0.207	1.176±0.123
	M	1.381±0.429*	1.500±0.295*	1.508±0.127*	1.124±0.332*	1.489±0.297*	1.446±0.481*

EC1: *Escherichia coli* ATCC 14169; EC2: *E. coli* ATCC 25922; EC3: *E. coli* ATCC 35218 (ESBL); SE: *Salmonella enterica* NCTC 6017; PA: *Pseudomonas aeruginosa* ATCC 27853.

E: ethanol; M: methanol.

Marked concentrations of extracts caused the change in the biofilm-forming capacity in comparison to the positive control.

*Decreasing of the biofilm-forming category or biofilm elimination.

**Increasing of the biofilm-forming category.

Table 4. TCP values after application of subinhibitory concentrations of extracts on *C. albicans*

Yeast	Solvent	Subinhibitory concentrations of extracts (µg/ml)				
		31.25	**15.63**	**7.81**	**3.90**	**1.95**
CA	E	0.078±0.008	0.084±0.014	0.085±0.016	0.086±0.015	0.090±0.024
	M	0.229±0.078	0.212±0.086	0.237±0.026	0.195±0.095	0.204±0.054

CA: *Candida albicans* ATCC 10231

E: ethanol; M: methanol.

the antimicrobial activity of the sweet cherry stem extracts (Ademović et al., 2017) showed that alcoholic extract possesses medium antibacterial activity against *S. aureus*, while there was no effect against *E. coli* and *C. albicans* even with the application of higher concentration of the extract (50 mg/ml). Obtained activity can be attributed to the large amounts of phenolic compounds detected in extracts. Furthermore, another investigation (Afonso et al., 2020) confirmed that methanolic extract of sweet cherry stems inhibits *S. aureus* and *E. faecalis*, but no activity against Gram-negative bacteria was noticed. These authors consider sakuranetin as the main antibacterial constituent, and mechanisms of its antibacterial activity comprise the formation of the complexes with proteins, inactivation of microbial adhesins, enzymes, and transport proteins, as well as the disruption of microbial membranes (Kumar and Pandey, 2013). Other studies on the microbiological activity of the sweet cherry extracts suggest that other plant parts aside from the fruit, such as bark, wood, leaves, and petioles also have the diverse antimicrobial potential (Rovčanin et al., 2015; Oyetayo and Bada, 2017; Ortega-Vidal et al., 2021). Different flavonoids found in the bark extract of *P. avium* can be associated with antimicrobial activity: dihydrowogonin, epitaxifolin, scopoletin, etc. (Abedini et al., 2020).

In general, Gram-positive bacteria are more susceptible to antimicrobial agents in comparison to Gram-negative bacteria, mainly due to the differences in their cell wall structure (Elisha et al., 2017). This study showed that sweet cherry stem extracts performed stronger inhibition of Gram-negative bacteria, which is in accordance with other investigations (Oyetayo and Bada, 2017) where sweet cherry extracts made from the leaves and stem bark were tested for antimicrobial activity. Sweet cherry stem bark extract tested earlier (Abedini et al., 2020) also did not perform the microbiocidal effects against all tested microbial strains. Nevertheless, considering the emerging antibiotic resistance and severity of infections caused by the investigated microbial strains, growth inhibition itself is encouraging in terms of further isolation and identification of active compounds from this plant species.

Two different life phases can be distinguished in the bacterial life cycle: planktonic (unicellular) and biofilm (multicellular), and according to the comparison of the different whole transcriptomes in some bacteria, each of them is associated with particular transcriptional behavior. Some genes such as those involved in iron-sulfur and lipid metabolism, amino acids and carbohydrate transport, biosynthesis of secondary metabolites, genes encoding efflux system compounds, and those involved in the stress response are up-regulated during biofilm formation. On the other hand, due to the down-regulation of DNA repair genes, there is an increased rate of spontaneous mutations in biofilms which leads to the

occurrence of novel genetic traits (Berlanga and Guerrero, 2016).

Different *S. aureus* strains included in this investigation showed diverse responses in the biofilm-forming capacity alteration when treated with sweet cherry stems extracts. Biofilm formation in *S. aureus* is regulated via the accessory gene regulator (*agr*) quorum sensing system, and the most prominent compound of these biofilms is so-called PIA (polysaccharide intercellular adhesin), the agent responsible for intercellular adhesion of the bacterial cells, as well as for the adhesion to external surfaces. This particular component is under the control of the intercellular adhesion (*ica*) locus; *icaADBC,* and *icaR* (biosynthetic and regulatory) genes. The *icaR* expression is associated with the Staphylococcal accessory regulator A (SarA), and Sigma B (σ^B), while the Rbf protein (Regulator of biofilm formation) is the negative regulator of *icaR* gene, which is related to the increased *ica* gene expression, production of the PIA and biofilm formation. Inhibition of the biofilm formation is achieved through the regulation of *icaADBC* via the *spx* gene, a stress response effector of *icaR*.

Different external factors have an impact on the *ica* locus (Parastan et al., 2020). A previous study (Abedini et al., 2020) noted inhibition of *S. aureus* biofilm formation in the presence of sweet cherry bark extract and suggest dihydrowogonin as the responsible compound, while reduction of the biofilm could be linked to the decreasing of the matrix production and reduction in the number of adhered bacteria.

E. faecalis and *B. subtilis* included in this research performed an increase in the biofilm-forming category after the application of the sweet cherry extracts. Although *E. faecalis* is normally associated with the human intestinal microflora, it is an opportunistic pathogen with increasing antibiotic resistance and an etiological agent of nosocomial infections (Kristich et al., 2004). Understanding the biofilm formation of *E. faecalis* is still challenging since there are many virulence factors related, but in general *agg* (*E. faecalis* aggregating substance) is associated with strong, while *cylA* (cytolysin A) with weak biofilm formation (Zheng et al., 2018). Further investigations are needed to define a particular compound of the extract involved in the change of the biofilm-forming capacity. *B. subtilis* is soil bacteria, and commensal species of the human gastrointestinal tract (Gingichashvili et al., 2017), that express two different life strategies including biofilm formation and swimming motility, according to the decision made at the individual cell level. Expression of the genes needed for biofilm formation, but not those for swimming motility allowed this bacteria to overcome unfavorable external conditions (Ryan-Payseur and Freitag, 2018). The process of biofilm formation in this species begins with the expression of matrix genes in response to some external signal, but since there are several signals and mechanisms that can be associated with the increased expression of extracellular matrix genes (Vlamakis et al., 2013), further studies are required for the identification of specific molecular mechanisms and signalization involved in the bacterial response to plant products and their particular compounds.

There are some findings of the increase of the biofilm formations after the application of the sweet cherry extracts. Sweet cherry bark extract increases the biofilm formation of *P. aeruginosa* in subinhibitory concentrations, which could be explained as the specific response of this versatile pathogen to the stressful condition illustrated through the more matrix production (Abedini et al., 2020). We can speculate that our results regarding EF and BS represent a similar phenomenon.

E. coli comprises some MDR strains, that are extremely pathogenic and involved in high rates of morbidity and mortality. Besides other virulent strains that cause different intestinal and

extraintestinal infections, there is a specific and genetically heterogeneous group of uropathogenic *E. coli* strains or UPEC. These isolates are persistent, resistant to many antibiotics, and potentially fatal, and their pathogenicity is based on different virulence factors and genes for toxins and colonization factors associated with genetic mobile elements (Beloin et al., 2008; Sharma et al., 2016).

According to Sharma et al. (2016), there are numerous genes involved in the biofilm formation of *E. coli*, encoding different proteins located in the cell's inner membrane, peripheral membrane, cytoplasm, or represent multipass membrane proteins. The most investigated biofilm genes of *E. coli* are: *csgD* (CsgBAC operon transcription regulatory protein regulates fimbriae production and positively affects biofilm formation and stress regulation), *hha* (haemolysin expression-modulating protein Hha repress the transcription of fimbrial genes and decrease biofilm formation), *bcsA* operon (cellulose synthase catalytic subunit catalyzes the cellulose formation), *pgaC* (poly-beta-1,6-N-acetyl-D-glucosamine synthase involved in the PGA polymer synthesis, which helps the adhesion of biofilm), *fimB* (regulatory protein-FimB regulates type 1 fimbriae production).

The utilization of phytotherapeutic remedies based on sweet cherry stems is a well-known practice in case of different urinary infections, potentially caused by this bacteria. Our study included three *E. coli* strains, along with the ESBL that is multidrug-resistant and generated different results in terms of biofilm formation under the activity of extracts. In this sense, the most intriguing findings are related to the response of EC2 strain which was in planktonic form in all replication of the positive control, while the applied extracts stimulate the bacteria to produce biofilm. In the case of other strains, the increase and the decrease of the biofilm-forming capacity were noted. It is known that some phytochemicals can cause the quorum sensing and the motility inhibition of *E. coli* by downregulating curli and prophage genes, suppressing the levels of RssAB and HNS of flagellar master operon *flhDC*, or repressing the multiple drug resistance and other genes (Sharma et al., 2016). A precise chemical profile of investigated extracts is needed to determine the potential quorum sensing and quorum quenching pathways in the biofilm formation of *E. coli*. Such information could be essential in the correct therapeutic use of sweet cherry preparations.

P. aeruginosa is considered a life-threatening bacteria, and the pathogenicity and severity of *P. aeruginosa* infections are related to its excellent biofilm-forming capacity (Crespo et al., 2018). Four separate quorum sensing pathways can be noted in the *P. aeruginosa*: Las, Rhl, PQS, and IQS, with the Las system being at the top of the hierarchy. The first two quorum sensing circuits are triggered by the increased cell density at the preliminary exponential growth phase, while the other two are activated later in the exponential growth phase. The most important genes involved in the QS pathways of *P. aeruginosa* are *lasI* synthase gene, *rhlR* and *rhlI* genes, as well as the *pqsR* and *pqsABCDH* genes.

Many substances of plant origin target the quorum sensing inhibition for eradication of the biofilm, as well as behave as the quorum quenchers for the *P. aeruginosa* biofilms (Thi et al., 2020). Our investigation confirmed the strong biofilm-forming potential of *P. aeruginosa* but also revealed that investigated extracts successfully inhibited biofilm formations in the manner described in the Results section. Results potentially suggest that some chemical compound present in the sweet cherry stems extract could have the quorum quenching features for *P. aeruginosa* biofilm. These outcomes are promising, especially since the World Health Organization listed *P. aeruginosa* as the priority for research and development of new antibiotics. *Salmonella* species form biofilms with various extracellular matrix compounds usually

resistant to many commonly used antimicrobial agents. The major regulatory factor in the control and integration of Salmonella biofilms is *csgD*. This is a transcriptional response regulator containing an N-terminal receiver domain with a conserved aspartate and a C-terminal LuxR-like helix-turn-helix (HTH) DNA-binding motif belonging to the FixJ/NarL family. In a genomic context, *csgD* is an integral part of the curli biosynthesis system (Steenackers et al., 2012). Examined extracts did not cause the change in the weak biofilm-forming capacity of investigated *Salmonella* strain, but further investigations are needed for the isolation and the characterization of particular compounds that can act as antibiofilm agents.

Bacteria in the biofilm are using many resistance mechanisms to antimicrobial agents, such as integrons, chemical change and/or damage of antibiotics, decreased antibiotic penetration, efflux pump, target protection, etc. In some biofilms, the presence of persister cells contributes to the expanding resistance to antimicrobials (Parastan et al., 2020).

It is found that extract made from the sweet cherry wood contains different bioactive compounds such as catechin, taxifolin, aromadendrin, pinocembrin, and tectochrysin with antibacterial activity against *Enterobacter* sp., where some of these constituents slightly inhibit the biofilm formation, while some of them also cause the increase in the biofilm-forming capacity at a particular concentration (Ortega-Vidal et al., 2021). These findings could be involved in the results gained by this study in terms of the extract activity towards the biofilm formation in Gram-negative bacteria.

C. albicans is one of the biggest biofilm producers within the genus (Chandra et al., 2001), with prominent resistance to antifungal agents (Cavalheiro and Teixeira, 2018). Biofilm formation of *C. albicans* is under the control of different genes that could be classified into several categories: genes required for hyphae production, genes involved in the response to the quorum sensing molecules, genes encoding cell wall proteins, etc. It is known that many of them encode predicted transcription factors, but signals that affect the activity of various biofilm regulators in *C. albicans* are not well understood (Finkel and Mitchell, 2011). Considering the emerging resistance, severity of infections caused by this species as well as the toxicity of some antifungal agents (Elisabeth et al., 2016), generated results on antifungal and anti - *C. albicans* biofilm activity of the investigated extracts are encouraging in terms of isolation and identification of new antifungal drugs of natural origin.

Conclusion

This investigation provides an insight into the antimicrobial and antibiofilm activity of sweet cherry stems extracts, since such products are widely used in traditional medicine. Investigated products have the ability to inhibit microbial growth, without the cause the death of microbial cells. Subinhibitory concentrations tested in order to define the impact on the biofilm formation revealed that sweet cherry stem extracts can decrease, and in some cases prevent the biofilm formation, but there are also findings on the increased biofilm-forming capacity. Therefore, future investigations are needed in order to define particular constituents related to the changes in microbial biofilms. This is particularly important in terms of the rationally and justified utilization of plant antimicrobial products.

Conflict of interest

Authors declare no conflict of interest.

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