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HOW TO ESCAPE 'THE ESKAPE PATHOGENS' USING PLANT EXTRACTS

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Метою *роботи було визначення вмісту біологічно активних речовин, а саме поліфенолів та антоціанів,в екстрактах плодів аличі, чорниці, йошти, черешні, сливи, червоної та чорної смородини, та дослідження впливу екстрактів плодів цих рослин на ріст та біоплівкоутворенняклінічних ізолятів "збудників ESKAPE"in vitro.*

Матеріали і методи. *Антибіотикорезистентність штамів клінічного походження, а саме Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter cloacae визначали методом дифузії Кірбі-Бауера. Вміст біологічно активних речовин визначали методом тонкошарової хроматографії. Вплив ягідних екстрактів на вищезгадані ізоляти вивчали методом їх сумісного культивування. Здатність клінічних ізолятів до утворення біоплівки вивчали за допомогою спектрофотометричного методу з використанням кристалічного фіолетового, в якості барвника.*

Результати. *Проаналізувавши результати чутливості до антибіотиків клінічних ізолятів, було встановлено, що вони стійкі до всіх антимікробних препаратів. Аналіз вмісту біологічно активних речовин екстрактів ягід показав, що вони містять велику кількість антоціанів та поліфенолів, та володіють антибактеріальними властивостями. Було встановлено, що ізоляти клінічного походження здатні до біоплівкоутворення, а відібрані нами екстракти ягід володіють здатністю інгібувати сформовані біоплівки ізолятами клінічного походження, а саме: Klebsiella pneumoniae та Pseudomonas aeruginosa.*

Висновки. *Досліджуючи антибактеріальні властивості антоціанів та поліфенолів, екстрагованих з ягід, можна зробити висновок, що вони здатні гальмувати ріст не лише виділених нами планктонних форм штамів, але й пригнічують утворені ними біоплівки*

Ключові слова: *ізоляти клінічного походження, біоплівки, екстракти їстівних рослин*

1. Introductions

Acronymically termed ESKAPE pathogens are able to escape the biological impact of antibiotics and collectively represent new paradigms in pathogenesis, transmission and resistance [1]. They are primarily known as agents of nosocomial diseases, those microbes that make the most detrimental complications to major diseases and often cause death [2].

In recent years ESKAPE Pathogens appeared to be among the most dangerous ones, with the highest frequency of isolations in current hospital conditions [3]. The strategy for their prevention and effective treatment became one of the crucial challenges not only for donatives, but also for other segments of the population. One of the intentions of the health care system is to solve this issue by deploying strong controlling over dissemination in the environment (including resistome) of the majority of ESKAPE Pathogens and prohibition for using antibiotics for animal breeding and some other methodology.

The first step was to specify the methods for researching antibiotic sensitivity of clinical and non-clinical isolates of opportunistic bacteria as confirmation of the feasibility of using and replenishment of EUCAST database and developing a newly created unified domestic electronic resource [4]. The above-mentioned strains do not show any sensitivity to most of the currently known antibiotic agents. One of the ways to overcome the existing situation so far is the creation and development of new antibiotics, which is a promising trend, though, in recent years, almost unknown. Another promising trend is the development of new antimicrobials of other (natural) origin, namely the use of bioactive substances (extracts), of not only medicinal but also edible plants. These promising antimicrobial compounds are also defined by antioxidant (anti-inflammatory) and antibiofilm-forming properties [5].

It is known, that polyphenolic compounds, including anthocyanins, have the antimicrobial effect against a relatively wide range of microorganisms [6]. Anthocyanins exhibit the antimicrobial effect through induced cell damage by the destruction of a cell wall, membrane, and intercellular matrix [7].

2. Literary review

Phenolic compounds are one of the most important bioactive substances that give fruits a relevant taste. Anthocyanins are pigments, responsible for the quality of fruit, and are markers of ripening, as most fruits accumulate anthocyanins only in the ripening phase [8]. Anthocyanins, glycosides of anthocyanidins are derivatives of phenolic compounds and are natural plant pigments that give a color to fruit from red to purple. Previous studies showed that the alkaloids that are present in berries have anti-diarrheal, antimicrobial and anti-

inflammatory properties [9, 10]. Today, there are many papers, devoted to the use of anthocyanins in the treatment of cancer [11, 12], the study of their biological properties [13]. The antioxidant activity of mahogany berries was studied and it was proved, that polyphenols, especially anthocyanins, are the main compounds, responsible for antioxidant properties of berries [14].

Flavonoids are powerful antioxidants, free radical scavengers, and metal chelators; they inhibit lipid peroxidation and exhibit different physiological effects: anti-inflammatory, anti-allergic, anticarcinogenic, antihypertensive, anti-arthritic and antimicrobial effects [15, 16].

3. The aim and objectives of the study

The aim of the work was to determine the content of biologically active substances, namely polyphenols and anthocyanins, in the extracts of cherryplum, jostaberries, blueberries, sweet cherries, plums, red and black currants, and to study the effect of these extracts on the growth and biofilm formation of clinical isolates of ESKAPE pathogens in vitro.

To achieve the aim, the following tasks were set:

1. To carry out the phytochemical screening of biologically active substances, namely to determine the gross content of anthocyanins and polyphenols in the extracts of berries and fruits of edible plants.

2. To investigate the ability of edible plant extracts to inhibit the growth of ESKAPE Pathogens.

3. To research the ability of edible plant extracts to inhibit biofilm formation by ESKAPE Pathogens.

4. Materials and methods

In our study, we used *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, Enterobacter spp*. opportunistic pathogenic bacteria strains.

The strains were isolated from patients of the Transcarpathian Regional Clinical Center for Neurosurgery and Neurology inpatient department and the Transcarpathian Regional Cardiology Health Center inpatient department according to methodological guidelines [17]. For strains, isolation and identification of microorganisms of different genera, selective and chromogenic media were used as well as the following test systems: OXI test, INDOLtest, (produced by «PLIVA Lachema Diagnostika s.r.o.» Czech Republic), ENTERO-test 24, STREPTOtest16, STAPHYtest16, (PLIVA-Lachema diagnostica s.r.o, Brno city, Czech Republic).

Antibiotic resistance of isolated strains

Susceptibility determination of strains: *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, Enterobacter spp*. to antibiotics was defined by the Kirby-Bauer diffusion method [18]. The effect of 56 modern antibiotics, most commonly used in medicine to control pathogenic and opportunistic pathogens, was studied.

Extraction of bioactive substances

Methanol-free extracts of red currant (Ribes rubrum), sweet cherries (Prunus avium), plums (Prunus domestica), jostaberry (Ribes x nidigrolaria), blueberries (Vaccinium myrtillus), black currant (Ribes nigrum), cherry plum (Prunus cerasifera) were obtained by vacuum evaporation.

We determined the quantitative content of polyphenols and anthocyanins in methanol extracts by thin-layer chromatography (TLCH). We prepared the extract from 50 g of each kind of berries and used for extraction 100 ml of methanol of 80 % concentration. To determine the gross content of biologically active substances, the optical density at 765 nm was measured and compared with a standard scale of gallic acid and cyanidin-3-rutinoside [19, 20].

In order to study the antibacterial properties of berries extracts of edible plants, we used the method of cultivation of the studied extracts with previously selected microorganisms: *S. aureus, K. pneumoniae, P. aeruginosa, Enterobacter spp.* The initial concentration of the selected strains was 1.5×10^8 CFU / ml (N_0). The obtained data were expressed as the logarithm of the number of surviving bacteria (N_t) to the initial number of bacteria (N_0) - lg (N_t / N_0) for a certain culturing time (4 h, 14 h, and 24 h). For a positive control, we used selected strains of bacteria 1.5×10^8 CFU / ml, and for a negative control, plant extracts were used.

To define the ability of clinical isolates to form biofilms, the suspensions of the tested microorganisms' strains were prepared according to McFarland with 0.5 of dullness, corresponding to a concentration of 1.5×10^8 CFU / ml. 5 ml of the prepared bacterial suspension were introduced into sterile testing tubes and cultured at 37 ° C for 5 days.

After incubation, the contents of the testing tubes were removed and 3.5 ml of distilled water and 350 μl of 1 % alcoholic solution of crystalline violet were added. The testing tubes were incubated for 45 min. at the room temperature.

After incubation, the dye was removed and the testing tubes were washed three times with distilled water. 4 ml of 96 % ethyl alcohol were added into washed testing tubes and left for 45 minutes at the room temperature. Thereafter, spectrophotometric measurements were performed at wavelengths of 630 nm and 492 nm. The optical densities were measured on an SF-46 spectrophotometer and a KFK-3 photoelectric colorimeter in quartz cells. Statistical processing of the results of experiments was carried out using a software OriginLab 2017 version 94E.

5. Results and discussion

We have analyzed the antibiotic sensitivity of ESKAPE Pathogens, isolated from patients of the Transcarpathian Regional Clinical Center for Neurosurgery and Neurology inpatient department and the Transcarpathian Regional Cardiology Health Center inpatient department. From the obtained data, it was obvious that *P. aeruginosa* isolates were the highly resistant strains. Only cefepime and vancomycin proved to be effective among 56 applied antibiotics (Table 1). The moderate resistance was observed in relation to gatifloxacin, levofloxacin, meropenem, ciprofloxacin, gentamicin, ceftriaxone. *K. pneumoniae* strains were also characterized by the high resistance. The strains were sensitive only to gentamicin and phosphomycin out of all antibiotics used. *E. faecalis* strains were susceptible to 6 out of 56 tested antibiotics, namely: nalidixic acid, ampicillin, netilmicin, rifampicin, ciprofloxacin, sparfloxacin.

S. aureus strains were susceptible only to gentamicin, meropenem, nalidixic acid, oleandomycin, ciprofloxacinum, streptomycin, fosfomycin, azithromycin, netilmicin. *A. baumannii* strains are also defined by the high polyresistance to the antibiotics.

Enterobacter cloacae strains were susceptible to levofloxacin, amikacin, gentamicin, aztreonam, ampicillin/sulbactam, netilmicin, rifampicin, ticarcilin, nalidixic acid, fosfomycin (200), ciprofloxacin.

Table 1

Antibiotic sensetivity of isolated strains from patients of the neurological department

No.	Antimicrobial agent	Abbreviation	Growth inhibition zone diameter (mm)±SD					
			Species / quantity	*Species / quantity*	*Species / quantity*	*Species / quantity*	*Species / quantity*	*Species / quantity*
			E. faecalis/10	*S. aureus /15*	*K. pneumoniae /14*	*A. baumannii /12*	*P.aeruginosa /13*	*E. cloacae /14*
1	Azithromycin	AZM^{15}	–	20±4	6±1.5	–	4±4.5	5±5
2	Aztreonam	AT^{30}	0	21±0.6	6±0.6	–		6±1
3	Amikacin	AK^{30}	–	16±0.58	0	9±0.58	0	21±1
4	Ampicillin	AMP^{10}	22±1	6±0.58	0	3±0.58	0	0
5	Ampicillin-sulbactam	A/S$^{10/10}$	6±1	7±1	6±0.58	0	12±0.57	8±2
6	Amoxiclav	AC^{30}	9±1.5	8±1	7±0.57	4±0.57	–	6±2
7	Amoxicillin	AMX^{10}	–	5±1.53	9±0.57	0	–	–
8	Amphotericin	AP^{100}	7±1.15	0	8±0.57	0	0	0
9	Vancomycin	VA^{30}	14±0.6	0	0	15±0.57	23±0.57	6±1
10	Gentamicin	GEN^{10}	9±1	21±1.53	21±	18±2	17±1.5	22±2.5
11	Gatifloxacin		9±0.6	16±1.53	0	19±2	16±1	6±
12	Doxycycline	DO^{30}	5±1.5	6±1.53	0	5±0.58	0	5±0.58
13	Erythromycin	E^{15}	8±0.6	17±1.15	0	6±0.57	0	0
14	Clindamycin	CD^{2}	6±1.4	4±1.15	0	6±0.57	0	7±1.5
15	Clarithromycin	CLR^{15}	9±2.9	16±3.5	0	8±2	0	0
16	Lincomicin	L^{10}	6±1	5±3.5	0	0	0	6±1
17	Levofloxacin	LE^{5}	0	4±1	12±	17±2.5	17±3	0
18	Meropenem	МПН	7±0.58	20±1.52	0	19±2	17±2.5	6±0.57
19	Moxifloxacin	MO^{5}	0	6±2.51	5±	16±2	0	6±0.57
20	Metronidazole	MT^{15}	5±0.58	6±2.51	8±1	9±0.57	2±0.57	2±1
21	Methilmicin	MET^{5}	13±3	18±0.57	7±	4±0.57	5±0.58	5±0.57
22	Nalidixic acid	NA^{30}	22±0.58	21±0.57	7±0.57	4±0.58	9±0.57	12±2
23	Netilmicin	NET^{30}	21±2.52	20±0.57	5±1	4±0.57	8±0.58	9±0.57
24	Nitroxoline	NO^{30}	7±2	5±3.5	6±0.58	5±1.15	3±1	8±0.57
25	Novobicin	NV^{30}	8±3	8±2.01	6±1.5	6±2.5	0	7±1
26	Oleandomycin	OL^{15}	9±0.57	20±1.53	8±0.58	4±0.58	0	7±0.58
27	Oxacillin	OX^{5}	10±0.57	9±2.52	8±0.57	0	0	6±0.58
28	Ofloxacin	OF^{5}	7±0.57	6±0.58	8±0.58	0	0	5±0.58
29	Benzylpenicillin	P^{10}	6±6	7±1.15	5±0.57	0	0	9±1.53
30	Pipedimic acid	PA^{30}	6±0.57	17±3	0	0	0	8±0.57
31	Polymyxin B	PB^{300}	12±0.57	5±2	16±2	6±0.57	0	16±0.57
32	Piperacillin	PI^{100}	10±0,57	5±0.58	0	6±0.57	0	9±0.57
33	Rifampicin	RIF^{5}	22±2	6±0.58	0	5±0.57	0	11±0.57
34	Streptomycin	S^{10}	16±0.57	22±1.53	0	5±0.25	10±0.5	12±0.57
35	Sparfloxacin	SPX^{5}	23±3	22±1	0	5±	7±0.5	8±2
36	Spiramycin	SR^{100}	13±2	17±3	0	18±0.57	0	6±0.57
37	Tetracycline	TE^{30}	12±0.57	8±1.53	5±0.57	7±1	0	11±0.57
38	Tobramycin	TOB^{10}	10±3	6±1.53	5±0.57	4±0.57	0	–
39	Ticarcillin	TI^{75}	15±0.57	5±1.53	0	14±0.57	13±0.57	6±1.53
40	Ticarcillin-clavulanic acid	TCC$^{75/10}$	17±1	0	7±1	8±1	0	–
41	Fosfomycin	FO^{50}	16±0.57	0	6±0.5	0	0	–
42	Fosfomycin	FO^{200}	19±0.57	21±2	22±2.5	5±0.57	7±0.57	15±0.57
43	Furozalidone	FR^{50}	4±3,5	17±1.15	8±1	4±0.57	4±0.57	5±0.57
44	Cefixime	CFM^{5}	12±1.53	0	9±0.57	4±0.57	5±0.57	4±0.57
45	Cefamandol	CEF^{30}	10±1.53	0	10±1	0		
46	Cefepime	CPM^{30}	8±1.53	0	0	0	22±2.5	6±1
47	Cefotaxime	CE^{10}	7±1.55	0	3±0.57	14±2	12±1.5	6±1.5
48	Ceftriaxone	CTR^{30}	10±1	0	5±0.5	16±1.53	18±1.53	6±0.57
49	Cefoperazone	CPZ^{75}	–	0	0	15±1.53	0	6±0.57
50	Cefazolin	CZ^{30}	12±2.5	0	0	8±	5±1.47	6±2
51	Cefpodoxime	CEP^{10}	10±0.57	0	5±0.57	7±0.53	9±0.57	0
52	Ceftazidime	CA^{30}	9±0.57	0	0	0	11±1	6±2
53	Ceftazidime-avibactam	CAC$^{30/10}$	7±0.57	0	5±0.57	8±0.57	7±0.57	9±0.57
54	Cefoxitin	CN^{30}	16±2.5	0	6±0.57	0	6±1.53	6±0.57
55	Cefuroxime	CXM^{30}	17±2	0	7±0.57	0	14±2	6±0.57
56	Ciprofloxacin	CF^{5}	22±2.5	0	6±0.57	7±0.57	4±0.57	6±0.57

Having analyzed the results of antibiotic sensitivity of strains, isolated from patients of the Transcarpathian Regional Cardiology Health Center inpatient department (Table 2), we found *E. faecalis* strains to be resistant to almost all antibiotics, so it can be concluded, that this strain is more resistant than strains, isolated from patients of the Transcarpathian Regional Clinical Center for Neurosurgery and Neurology. *A. baumannii* strains were not found among the isolated strains. This can be explained by the fact that different types of disinfectants are used to disinfect the premises of the inpatient departments of Neurosurgery and Neurology Clinical Center and Cardiology Health Center. As part of BacFoodNet project, it was revealed, that one of the effective methods of controlling the polyresistance of nosocomial strains is the use of Cold Plasma Treatment [21].

Therefore, the use of antibiotic drugs is limited due to the polyresistance of isolated microorganisms that are related to nosocomial pathogens.

Table 2

Antibiotic sensitivity of isolated strains of patients from the cardiology health center

No.	Antimicrobial agent	Abbreviation	Growth inhibition zone diameter (mm) ±SD				
			Species / quantity	*Species / quantity*	*Species / quantity*	*Species / quantity*	*Species / quantity*
			E. faecalis /13	*S. aureus /15*	*K. pneumoniae / 10*	*P. aeruginosa / 15*	*E. cloacae/ 13*
1	Ampicillin	AMP^{10}	12±2	6±1.53	0	0	0
2	Ampicillin / sulbactam	$A/S^{10/10}$	6±1.53	6±1.15	6±0.57	12±2	3±1.15
3	Amoxiclav	AC^{30}	9±1	9±2	8±1.53	5±1.15	6±1.15
4	Bacitracin	B^{10U}	8±2	5±1.15	8±1.53	6±1.53	0
5	Vancomycin	VA^{30}	10±0.58	0	0	3±0.58	0
6	Gatifloxacin		9±0.58	6±1.15	0	7±0.58	0
7	Doxycycline	DO^{30}	5±1.53	6±1.15	6±1.15	0	5±1.15
8	Erythromycin	E^{15}	8±2.52	7±1.15	0	9±1.15	0
9	Clindamycin	CD^{2}	6±1.73	4±0.58	5±1	0	7±1.15
10	Clotrimazole	CC_{10}	4±1.15	5±1.15	8±0.58	5±1.52	3±0.58
11	Imipen		4±2	9±1.15	5±1.52	3±1.15	0
12	Levofloxacin	LE^{5}	0	4±0.58	3±1.15	3±1.15	0
13	Meropenem	МПН	5±1.52	10±1.52	0	7±1.15	6±0.58
14	Netilmicin	NET^{30}	3±1.15	10±1.15	5±0.58	8±1.15	9±1.15
15	Nystatin	NS^{100U}	11±0.58	12±1.52	9±1.53	9±0.58	8±1.52
16	Oxacillin	OX^{5}	10±1	9±	8±1.53	0	0
17	Pipemidic acid	PA	3±0.58	7±0.58	8±0.58	9±0.58	5±1.52
18	Rifampicin	RIF^{5}	4±1.52	6±	0	8±1.53	3±1.15
19	Streptomycin	S^{10}	6±1.15	12±0.58	0	10±1.53	0
20	Tetracycline	TE^{30}	8±1	8±1.52	5±1.15	0	8±1.53
21	Ticarcilin	TI^{75}	5±0.58	5±1.53	0	13±1.52	6±1
22	Fosphomycin	FO^{200}	4±1	11±0.58	12±0.58	7±1.53	6±0.58
23	Furozalidone	FR^{50}	3±1	17±1.52	8±1.53	4±1	0
24	Cefotaxime	CE^{10}	7±1.15	0	3±1	12±1.52	9±1.52
25	Ceftriaxone	CTR^{30}	3±1.53	0	5±1.53	6±1.5	0
26	Cefoperazone	CPZ^{75}	6±1.15	0	0	0	6±1
27	Cefazolin	CZ^{30}	0	0	0	5±1	0
28	Ciprofloxacin	CF^{5}	6±1.15	0	6±1	4±1.52	6±1

The gross content of anthocyanin and polyphenol compounds was defined in methanolic berry extracts by thin-layer chromatography. According to the results of the study, presented in Table 3, the highest content of polyphenols and anthocyanins was detected in the blueberry extract. The high content of anthocyanins and polyphenols was also found in the black currant extract, this can be explained by the fact that these berries have a dark color, which is provided by anthocyanins. Significantly less content of anthocyanins and polyphenols was found in jostaberry, red currant, plum, and sweet cherry extracts. The determination of anthocyanins and polyphenols gross content in the cherry plum extract showed no anthocyanins in this extract. It contains polar phenols, such as catechin and flavonoids.

Table 3

Anthocyanins and polyphenols gross content in berries extracts

No.	Extracts	Level of total anthocyanins, microg ekv. cyanidin-3-rutinoside/ml of extract	Level of total polyphenolics, microg ekv. galic acid/ml of extract
1	Cherry plum	–	219
2	Jostaberry	560.0	3250
3	Sweet cherry	78.5	668
4	Black currant	2886.2	5042
5	Red currant	178.8	3962
6	Blueberry	4779.277	8945
7	Plum	18.8	525

Taking into account the results of gross content of anthocyanins and polyphenols in methanol extracts, we researched the ability of these extracts to inhibit the growth of opportunistic pathogens. For this purpose, non-methanol extracts of berries were obtained by vacuum evaporation, which were used to study their antibacterial properties.

We revealed (Table 4) that after 4 hours of incubation the cherry plum extract had the antimicrobial effect against *K. pneumoniae, E. cloacae, P. aeruginosa, S. aureus*, the number of which significantly decreased. After 14 hours of incubation, no growth was observed in all strains, tested by us, except for *K. pneumoniae*, the number of which decreased. After 24 hours of extracts cultivation with selected strains the bacterial growth was absent.

4-hour incubation of the sweet cherry extract with the tested microorganisms' strains showed the low antagonistic effect, compared to the cherry plum extract. The studied extract showed the antibacterial effect against *E. cloacae, S. aureus* strains, but *P. aeruginosa* strains were not susceptible to the impact of the sweet cherry extract.

After 14 hours this extract showed the antibacterial effect to all tested microorganisms. There was no growth of *E. cloacae* and S. *aureus* microorganisms. After 24 hours of incubation no growth was observed in all microorganisms.

In the combined cultivation of the plum extract with clinical isolates for 4 hours no antagonistic effect was observed against *P. aeruginosa* strain. However, the other strains were susceptible to the impact of the extract. After 14 hours of incubation, there was no growth of *S. aureus, E. cloacae, K. pneumoniae*. The number of other microorganisms' strains decreased significantly. After 24 hours of incubation the growth of all strains was absent.

After 4 hours of incubation, the antagonistic effect of jostaberry extract in *P. aeruginosa* was not observed. Other strains revealed the antibacterial effect of the extract. There was no growth of *S. aureus* and *E. cloacae* after 14 hours of incubation. Other strains showed the antibacterial effect of the extract. After 24 hours, the number of tested microorganisms did not significantly decrease, compared to the data, obtained after 14 hours of incubation. The lack of growth is observed in *P. aeruginosa, S. aureus* only.

The low antibacterial effect was shown by the red currant extract against *E. cloacae* and *K. pneumoniae*.

After 14 hours of incubation, the number of microorganisms changed significantly. No growth was observed in *P. aeruginosa* and *K. pneumoniae*, and no growth of *S. aureus* was observed after 24 hours of incubation, in addition to the above-mentioned bacteria.

After 4 hours of incubation, the weak antibacterial effect was shown by blueberry extract against *E. cloacae*. The number of microorganisms remained almost unchanged in the subsequent incubation periods – 14 and 24 hours, but was still less, compared to a similar one during 4-hour incubation. These extracts demonstrated the antagonistic effect on all tested strains.

Table 4

Biological influence of the fruit and berry extracts on the growth of Eskape Pathogens, in dynamics

	K. pneumoniae			*P.aeruginosa*			*S. aureus*			*E. cloacae*		
Treatment time, h	24	14	4	24	14	4	24	14	4	24	14	4
Cherry plum	–**	–6.5± ±0.29*	–5.2± ±0.29*	–**	–**	–2.5± ±0.38*	–**	–**	–3.13± ±0.55*	–**	–**	–4.17± ±0.58
Jostaberry	–6.5± ±0.5	–4.17± ±0.5	–2.5± ±0.14	–**	–6.5± ±0.23	–0,5± ±0.29	–**	–**	–4.17± ±0.27	–**	–**	–4.17± ±0.29
Sweet cherry	–**	–4.47± ±0.14	–2.47± ±0.29	–**	–6.47± ±0.29	–0.47± ±0.14	–**	–**	–4± ±0.38	–**	–**	–5.17± ±0.28
Black currant	–**	–4.47 ±±0.29	–4± ±0.76	–**	–6.47± ±0.29	–0.47± ±0.29	–**	–**	–2± ±0.25	–3.17± ±0.25	–2.17± ±0.29	–2.17± ±0.5
Red currant	–**	–**	–2.47± ±0,76	–**	–**	–4.47± ±0.29	–**	–6.47± ±0.14	–5.17± ±0.29	–3.17± ±0.25	–2.17± ±0.29	–2.17± ±0.5
Blueberry	–**	–4.47± ±0.29	–3.87± ±0.58	–6.47± ±0.29	–6.47± ±0.25	–6.47± ±0.29	–**	–6.47± ±0.14	–4.36± ±0.76	–3.17± ±0.25	–2.17± ±0.3	–2.17± ±0.25
Plum	–**	–**	–** 4±0.25	–**	–** 4.47± ±0.29	–** 0.47± ±0.29	–**	–**	–6.47± ±0.29	–**	–**	–5.17± ±0.29

Note: Significant differences with the cherry plum extracts on growth of Eskape Pathogens by (P<0.05); ** – bacteria growth is absent*

The most frequently isolated strains were *K. pneumoniae* and *P. aeruginosa*, accounting for up to 70 % of selection frequency. Considering the fact that most bacteria do not exist in free-floating state but in formed associations – biofilms, we researched the ability of *K. pneumoniae* and P. *aeruginosa* strains, which did not show an antibiotic sensitivity, to form biofilms.

Defining the optical density of *K. pneumoniae* and *P. aeruginosa* strains biofilms at the optical density of 630 nm showed that for the first day of cultivation the values of the formed biofilm were in the range from 0.039 to 0.026 units of optical density (UN OD). The greatest ability to form biofilms was observed in *P. aeruginosa* – 0.039±0.001. *K. pneumoniae* biofilms were significantly less, but also at the high level – 0.026±0.003. Such an optical density indicates that biofilm has begun to form. Starting on the second day, the optical density of sample biofilms increased, which was also observed during the third and fourth incubation days. The maximum optical density was reached on the fifth day of cultivation and made up 0.240±0.012 for P. *aeruginosa* and 0.135±0.005 for *K. pneumoniae*, respectively. Analyzing the data, we came to the conclusion that the formation of a biofilm begins on the first day of incubation.

Table 5

The ability to biofim formation by *P.aeruginosa, K. Pneumoniae* at the optical density of 630 nm

Bacteria	24 hours, UN OD±SD	48 hours, UN OD±SD	72 hours, UN OD±SD	96 hours, UN OD±SD	120 hours, UN OD±SD
P.aeruginosa	0.039±0.001	0.075±0.006	0.186±0.005	0.236±0.03	0.240±0.012
K. pneumoniae	0.026±0.003	0.045±0.007	0,118±0.005	0.128±0.001	0.135±0.005

When analyzing the results, obtained at the wavelength of 492 nm, as a whole, the values were lower than those, obtained at the wavelength of 630 nm. The range of values for P. *aeruginosa* was 0.023, and for *K. pneumoniae* – 0.016 UN OD on the first day of incubation. Their maximum was reached on the fifth day of cultivation.

The final stage of our study was to prove the antimicrobial ability of the extracts of edible plants to inhibit the growth of biofilms, formed by *P. aeruginosa* and *K. pneumoniae* strains.

Comparing the values of the optical density of the formed biofilm and the measurement results after the impact of the extract on the biofilms, we observed significant differences, compared to planktonic forms. Thus, for *P. aeruginosa* strains, the optical density decreased to 0.019±0.005 UN OD, and for *K. pneumoniae* – to 0.001±0.001 UN OD (Table 7).

Therefore, it can be concluded, that all the researched extracts have antibacterial properties against biofilm formation.

Table 6

The ability to biofim formation by *P.aeruginosa, K. Pneumoniae* at the optical density of 492 nm

Bacteria	24 hours, UN OD±SD	48 hours, UN OD±SD	72 hours, UN OD±SD	96 hours, UN OD±SD	120 hours, UN OD±SD
P. aeruginosa	0.023±0.005	0.035±0.003	0.110±0.003	0.186±0.004	0.19±0.005
K. pneumoniae	0.016±0.003	0.034±0.003	0.067±0.005	0.088±0.006	0.102±0.005

Table 7

The ability of dibble plant extracts to inhibit biofilm formation *P. aeruginosa, K. pneumoniae*

Bacteria	*P.aeruginosa,* OD±SD		*K. pneumoniae,* OD±SD	
Extracts	Control	After treatment	Control	After treatment
Cherry plum	0.240±0.012	0.019±0.01*	0.135±0.005	0.001±0.005*
Jostaberry	0.240±0.012	0.022±0.008	0.135±0.005	0.015±0.008
Sweet cherry	0.240±0.012	0.015±0.005	0.135±0.005	0.013±0.005
Black currant	0.240±0.012	0.009±0.004	0.135±0.005	0.005±0.003
Red currant	0.240±0.012	0.017±0.006	0.135±0.005	0.010±0.005
Blueberry	0.240±0.012	0.014±0.005	0.135±0.005	0.008±0.005
Plum	0.240±0.012	0.011±0.009	0.135±0.005	0.007±0.003

The results were expressed as the mean ± standard deviation (SD) (*P<0.05) significant differences with the cherry plum extract on biofilm formation by P. aeruginosa, K. pneumoniae.

6. Discussion

The literature data analysis showed that the activity of polyphenols against gram-negative bacteria is higher, compared to gram-positive strains due to the outer membrane of gram-positive bacteria, which acts as a barrier to permeability, which leads to a decrease in the absorption of compounds into a cell. The mechanisms that are responsible for the toxicity of pure phenolic compounds to microorganisms include inhibition of enzyme by oxidized compounds, possibly through the reaction with sulfhydryl groups or through more nonspecific interactions with proteins that lead to their inactivation and loss of function. However, analyzing the obtained experimental data, we found that the cherry plum extract, in which anthocyanins were not found, but flavonoids were present, inhibited the growth of both gram-positive and gram-negative bacteria. This extract showed the best antibacterial effect. Analyzing the obtained experimental data on antibacterial properties of jostaberry, sweet cherry, blueberry, plum, black currant and red currant extracts against the tested strains of microorganisms, virtually all strains proved to be susceptible, both gram-positive and gram-negative. They inhibited the growth of both gram-positive (*S. aureus, E. cloacae*) and gram-negative microorganisms (*K. pneumoniae, P. aeruginosa*). However, there is little information on the antimicrobial ability of phenols present in berries. In our studies, anthocyanin-rich extracts inhibited gram-negative and gram-positive bacteria.

All the data, we obtained earlier, and the results of the studies in this paper demonstrate that the cherry plum extract possesses the best antibacterial effect. Other extracts also have a significant ability to inhibit the growth of microorganisms belonging to ESKAPE Pathogens, which makes them promising antibacterial agents.

Antibacterial agents of biological origin in aerosol form can be used in medicine.

The disadvantage of edible plant extracts is their limited shelf life due to the possibility of oxidation of biologically active compounds.

7. Conclusions

1. The phytochemical analysis has revealed a high content of polyphenols and anthocyanins in the methanol extracts of edible fruits (cherry plum, jostaberry, blueberry, cherry, plum, red and black currants). The highest content of aforementioned phytochemicals was registered in the blueberry, the lowest one - in the plam.

2. Methanol extracts of the investigated edible fruits exert the inhibitory effect on the growth of clinical isolates of ESKAPE pathogens. Most pronounced inhibitory effect was revealed for the extracts of cherry plum and plum, which contain the lowest amount of polyphenolics and anthocianins.

3. Extracts of investigated edible fruits also inhibit biofilm formation by clinical isolates of ESKAPE pathogens. The most powerful inhibitory effect was registered for the extracts of black currant and cherry plum. Taking into account the safety of edible fruit extracts, they could be considered as promising antimicrobial agents.

Conflict of interest

There is no conflict of interest

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