Evaluation of Fruit Extracts Influence on the Susceptibility of Escherichia Coli Rods to the Bactericidal Action of Human Serum

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Abstract

This study determined the influence of the methanol (ME) and water (WE) fruit extracts obtained from eight species of *Rosaceae* and *Grossulariacae* family on the susceptibility of *Escherichia coli* rods to the lytic action of normal human serum (NHS). Bacteria were incubated for 24 h in tryptic soy broth with varying concentrations (1, 5, 10, 20, 30, 40, and 50 mg ml-1) of raspberry, cherry, hawthorn, dog rose, gooseberry, chokeberry, quince, and Japanese quince extracts and then the bactericidal activity of NHS was established. We found that the resistance of *E. coli* rods to the bactericidal action of serum was altered by prior incubation with all tested extracts and was dependent on plant extract concentration. Among the tested extracts, gooseberry (both ME and WE), raspberry ME and cherry WE were responsible for the most profound changes in serum resistance of *E. coli* rods. Evaluation of the antimicrobial mechanisms of action of phenolics-rich plant extracts has the potential to impact the development of novel compounds with promising applications in food and biopharmaceutical industry or medical approaches to preventing and treating pathogenic infections.

Introduction

For centuries, humans have used plants to treat infections and other medical conditions. A wide variety of plant extracts have been found to exert a beneficial effect on human health, including those recommended as a dietary supplement which are available over the counter in the form of single compounds or mixtures. For instance, phenolic compounds found in numerous fruits and berries may enhance cardiovascular fitness and act as strong antioxidant, anti-inflammatory, antiproliferative and anticarcionogenic agents (Cowan 1999; Cisowska et al. 2011; De Pascual-Teresa and Sanchez-Ballesta 2008). Importantly, previous studies indicate that plant phenolics also possess antimicrobial activity against human pathogens. Both Gram-negative and Gram-positive bacterial strains are selectively inhibited by phenolic compounds of various berries by diverse mechanisms (Puupponen-Pimiä et al. 2005). It was noted that cloudberry and raspberry were the best inhibitors of bacterial growth and that *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella enterica* sv. Typhimurium and *Salmonella enterica* sv. Infantis strains were the most sensitive microbes. Nohynek et al. (2006) observed that phenolic extracts of cloudberry and raspberry, but not anthocyanin fractions of raspberry, disintegrated the outer membrane of *S. enterica* sv. Typhimurium and *S. enterica* sv. Infantis rods. Antimicrobial activity of berries may also be related to anti-adherence of bacteria to epithelial cells, which is a prerequisite for colonization and infection of many pathogens (Guay 2009). Such correlation was demonstrated by Liu et al. (2008) for compounds of cranberry juice, which were shown to disrupt bacterial ligand binding to uroepithelial cell receptor and decrease bacterial attachment to these cells. Exposure to cranberry juice also decreased the average length of Pfimbriae produced by *Escherichia coli* rods. Gene expression analysis confirmed the down regulation of flagellar basal body rod and motor proteins of P-fimbriae of *E. coli* strain incubated in the presence of proanthocyanidins (PACs) rich cranberry (*Vaccinium macrocarpon*) juice or extract (Johnson et al. 2008). In particular, the A type PACs in cranberry were shown by Howell (2007) to prevent primarily adherence of P-fimbriated uropathogenic *E. coli* to uroepithelial cells *in vitro*. Ingestion of cranberry products not only significantly reduced P-fimbriated uropathogen adherence to uroepithelium, but also decreased the surface hydrophobicity and biofilm production of *E. coli* rods (Uberos et al. 2011).

Apart from cranberries, various other fruits contain ample amounts of phenolic compounds. Previously, we determined the phenolic and anthocyanin content as well as antioxidant, potential anti-inflammatory properties and antimicrobial activity of the fruit extracts obtained from eight species of *Rosaceae* and *Grossulariacae* family (Strugała et al. 2015; Hendrich et al. 2020). Specifically, it has been shown that blackcurrant, chokeberry, blackberry and Japanese quince extracts significantly reduced adhesion of *E. coli* rods to epithelial cells; in contrast, gooseberry was the most active inhibitor of biofilm formation. Furthermore, it was also established that none of the tested plant extracts affected the

curli and P fimbriae production as well as hydrophobic properties of the surface of the bacterial cells. In the course of the present study, we investigated the influence of these fruit extracts on susceptibility to serum of *E. coli* rods.

Materials And Methods

Bacterial strain and growing conditions

Escherichia coli 192 strain, freshly isolated from the urine of patient with urinary tract infection, was used. A stock culture was kept at 4ºC on nutrient agar plate (BIOMED, Warsaw, Poland) and before each experiment bacteria were transferred to tryptic soy agar and incubated at 37ºC for 18 h. Afterwards, *E. coli* rods were incubated in tryptic soy broth (BIOCORP, Warsaw, Poland) with varying concentrations (1, 5, 10, 20, 30, 40, and 50 mg mL^{-1}) of methanol (ME) and water (WE) extracts of raspberry, cherry, hawthorn, dog rose, gooseberry, chokeberry, quince, and Japanese quince for 24 h at 37ºC. Control cultures contained bacteria untreated with examined fruit extracts.

Plant materials

Raspberry (*Rubus idaeus* L.) fruits, of Polka variety; cherry (*Prunus cerasus* L.), Łutówka variety; hawthorn (*Crataegus monogyna* Jacq.) and dog rose (*Rosa canina* L.), fruits collected in Szczytnicki Garden in Wrocław; gooseberry (*Ribes uva-crispa* L.) Red Triumf variety; chokeberry {*Aronia melanocarpa* (Michx.) Elliott} fruits of the Galician variety, picked from the Sady Trzebnickie plantation; quince (*Cydonia oblonga* Mill.), fruits obtained in Arboretum and Institute of Physiography in Bolestraszyce, and Japanese quince (*Chaenomeles speciosa* (Sweet) Nakai), fruits collected in Szczytnicki Garden in Wrocław were chosen for study. Preparation of the methanol (ME) and water (WE) extracts was previously described by Sroka et al. (1994); additionally, phenolic and anthocyanin content of these fruit extracts has been determined recently (Strugała et al. 2015).

Serum

Normal human serum (NHS) was obtained from healthy adult volunteers untreated with any antimicrobial drugs. The samples of NHS were collected, pooled, and stored in 0.25 mL portions at − 22ºC. A suitable volume of the serum was thawed immediately before experiments and each portion was used only once.

Antimicrobial activity of the plant extracts

Antibacterial activities of the tested plant extracts were determined by the standard dilution method. After 24-hour incubation at 37ºC with different concentrations of ME and WE fruit extracts *E. coli* rods were centrifuged (2500 g, 20 min), washed 3 times in phosphate buffered saline (PBS) and cultured overnight on nutrient agar plates at 37ºC. The influence of examined extracts on bacterial growth was assessed on the basis of the number of colony forming units per one milliliter (CFU mL^{-1}).

Bactericidal activity of serum

The bactericidal activity of NHS was determined as described previously (Cisowska and Bugla-Płoskońska 2014). Briefly, *E. coli* 192 strain was incubated in tryptic soy broth at 37ºC for 24 h with or without (control) varying concentrations of tested fruit extracts and afterwards bacterial cells were centrifuged (2500 g for 20 min) and washed 3 times in PBS. Subsequently, 0.25 mL of bacterial suspension was added to equal amount of NHS giving the final serum concentration of 50%. Bacteria with NHS were incubated in a water bath at 37ºC. The samples were collected after 0, 60, and 180 min and then bacteria were immediately diluted and cultured on nutrient agar plates for 18 h at 37ºC. The number of colony forming units at 0 min was assumed as 100%. *E. coli* rods with a survival ratio of more

than 50% after 180 min of incubation in the serum were regarded as resistant. The mean values from three separate experiments were calculated.

Results

Using the serial dilutions method, we determined the antimicrobial activities of the methanol and water fruit extracts of eight selected species belonging to the *Rosaceae* and *Grossulariacae* family (Table 1). Comparison of the results showed that with the exception of hawthorn and dog rose (both ME and WE) and raspberry ME, all other examined methanol and water extracts inhibited the growth of *E. coli* strain compared with the control. Raspberry, cherry, gooseberry, chokeberry, quince, and Japanese quince ME and WE varied in effectiveness and their antibacterial action also depended on the concentration of the extract.

Table 1
Effects of methanol (ME) and water (WE) fruit extracts on *E. coli* 192 strain growth, as observed after 24 h incubation.

Extract			**1**	**5**	**10**	**20**	**30**	**40**	**50**
Concentration [mg/mL]									
colony forming units [CFU/mL^{-1}]	raspberry	ME	2.0×10^{9}	1.3×10^{9}	6.8×10^{8}	8.1×10^{8}	3.8×10^{8}	2.5×10^{8}	2.0×10^{8}
		WE	9.9×10^{8}	4.5×10^{8}	1.0×10^{8}	4.2×10^{6}	1.3×10^{6}	2.1×10^{6}	1.2×10^{6}
	cherry	ME	1.6×10^{9}	2.9×10^{9}	3.7×10^{9}	6.7×10^{7}	5.3×10^{7}	2.0×10^{7}	1.9×10^{5}
		WE	7.3×10^{8}	8.4×10^{8}	2.4×10^{8}	6.1×10^{7}	9.8×10^{5}	8.9×10^{4}	8.9×10^{3}
	hawthorn	ME	8.8×10^{8}	1.3×10^{9}	2.0×10^{9}	1.6×10^{9}	1.5×10^{9}	1.1×10^{9}	1.0×10^{9}
		WE	9.8×10^{8}	9.0×10^{8}	9.6×10^{8}	4.9×10^{8}	5.7×10^{8}	4.0×10^{8}	5.5×10^{8}
	dog rose	ME	1.5×10^{9}	5.3×10^{9}	1.4×10^{9}	4.8×10^{8}	1.0×10^{8}	1.0×10^{8}	3.2×10^{7}
		WE	1.7×10^{9}	1.6×10^{9}	9.2×10^{8}	8.0×10^{8}	1.2×10^{9}	1.0×10^{8}	5.0×10^{8}
	gooseberry	ME	1.0×10^{9}	1.1×10^{9}	1.2×10^{9}	1.5×10^{8}	9.4×10^{6}	1.4×10^{6}	7.9×10^{4}
		WE	9.0×10^{8}	2.9×10^{8}	3.1×10^{7}	2.8×10^{7}	3.1×10^{7}	1.8×10^{7}	2.7×10^{7}
	chokeberry	ME	1.0×10^{9}	9.5×10^{8}	8.6×10^{8}	1.4×10^{8}	7.3×10^{7}	9.8×10^{7}	1.3×10^{6}
		WE	8.7×10^{8}	2.4×10^{9}	1.9×10^{9}	9.3×10^{6}	3.6×10^{6}	1.8×10^{4}	1.9×10^{4}
	quince	ME	7.8×10^{8}	9.9×10^{8}	8.7×10^{8}	3.1×10^{8}	9.8×10^{7}	3.0×10^{6}	2.3×10^{5}
		WE	6.4×10^{8}	6.0×10^{8}	5.7×10^{8}	1.4×10^{8}	1.4×10^{8}	2.6×10^{7}	1.0×10^{7}
	Japanese quince	ME	2.4×10^{8}	2.8×10^{8}	2.2×10^{3}	2.5×10^{2}	1.5×10^{1}	4.0×10^{1}	1.5×10^{1}
		WE	9.8×10^{8}	9.2×10^{8}	2.3×10^{6}	4.4×10^{4}	2.1×10^{3}	4.0×10^{3}	5.8×10^{2}
control			8.9×10^{8}						

In the majority of assessed fruits, 1, 5 and 10 mg mL^{-1} concentrations of ME and WE had no influence on the survival of the tested microorganism and the values of CFU mL^{-1} were similar to control (approximately 10^8-10^9 bacteria per ml). At higher concentrations, the number of *E. coli* rods in the cultures gradually decreased (quince ME, chokeberry WE, gooseberry ME, and both cherry extracts) or values of CFU mL^{-1} were only slightly reduced (quince WE, gooseberry WE, chokeberry ME, and raspberry WE). Only Japanese quince was found to be an efficient bacterial growth inhibitor because both its extracts in concentrations ranging from 10 to 50 mg mL^{-1} significantly reduced the number of bacterial cells (ME approximately 10^3-10^1 CFU mL^{-1} and WE 10^6-10^2 CFU mL^{-1}, respectively). Hawthorn ME and WE, raspberry ME and dog rose WE had no impact on survival of *E. coli* rods. For all concentrations (from 1 to 50 mg mL^{-1}), the values of CFU mL^{-1} were comparable to control. A similar effect was observed for methanol dog rose extract since only incubation at a concentration of 50 mg mL^{-1} slightly inhibited the growth of bacteria (3.2×10^7 CFU mL^{-1}).

The results concerning the bactericidal activity of 50% NHS against *E. coli* rods, which had been previously incubated for 24 hours in the presence of methanol and water fruit extracts are presented in Table 2. *E. coli* 192 strain that has been selected for the research is resistant to bactericidal action of human serum. The survival ratio of bacteria that had not been treated with any of the studied ME and WE after 180 min of incubation in serum was 132.6 percent of initial cell number. As can be seen in Table 2, the susceptibility of the serum resistant *E. coli* strain to the bactericidal action of NHS was altered by a previous incubation of these rods with all tested extracts, although this effect depended on their concentration. The most spectacular changes were recorded for both methanol and water gooseberry extracts. Specifically, we found that NHS decreased the percent survival of *E. coli* rods to 18.4-0.003 after 180 min of incubation for all tested ME and WE concentrations. The similar effect on the survival of bacteria in serum was recorded for raspberry ME and cherry WE, although at the lowest concentrations (1–10 mg mL^{-1}) *E. coli* rods were killed slightly less effectively (45.2–14.4% and 50.0–29.0% of initial cell number, respectively). Both methanol and water quince, water hawthorn and raspberry extracts showed modest action as bacteria remained resistant to NHS only at 1–5 mg mL^{-1} concentration, but were killed efficiently after the exposure to higher concentrations of these extracts (36.5-0.005%, 0.5-0.009%, 50.0-0.01% and 4.7 – 0.1% of initial cell number, respectively). In addition, similar effect was also observed for Japanese quince ME and WE at concentrations from 1 to 10 mg mL^{-1}. *E. coli* strain was resistant to the lytic activity of serum only after exposure to 1 mg mL^{-1} of these extracts. Moreover, seeing that Japanese quince ME and WE, in concentrations ranging from 20 to 50 mg mL^{-1}, significantly reduced the number of bacteria cells observed after 24 hours of incubation (approximately 10^3-10^2 CFU mL^{-1}) we did not examine the susceptibility of these bacteria to the action of NHS.

Table 2 Bactericidal activity of 50% NCS against *E. coli* 192 rods, which had been previously incubated for 24 hours in the presence of methanol (ME) and water (WE) plant extracts.

Extract		methanol (ME) plant extracts				water (WE) plant extracts			
Concentration [mg mL^{-1}]		Time of incubation in serum (min)			survival % after 180 min	Time of incubation in serum (min)			survival % after 180 min
		0	60	180		0	60	180	
		colony forming units [CFU mL^{-1}]				colony forming units [CFU mL^{-1}]			
Raspberry	1	1.8×10^{9}	3.4×10^{8}	2.6×10^{8}	14.4	6.5×10^{8}	2.4×10^{8}	4.8×10^{8}	73.8
	5	7.8×10^{8}	3.7×10^{8}	2.7×10^{8}	34.6	2.2×10^{8}	1.5×10^{8}	2.6×10^{8}	118.2
	10	6.2×10^{8}	3.7×10^{8}	2.8×10^{8}	45.2	9.2×10^{7}	3.7×10^{7}	4.3×10^{6}	4.7
	20	4.4×10^{9}	2.6×10^{8}	1.4×10^{8}	3.2	1.4×10^{6}	8.9×10^{4}	3.5×10^{4}	2.5
	30	3.9×10^{9}	1.6×10^{7}	3.9×10^{7}	1.0	1.2×10^{6}	6.8×10^{2}	4.2×10^{3}	0.4
	40	2.0×10^{9}	1.1×10^{6}	2.7×10^{6}	0.1	1.1×10^{8}	4.4×10^{5}	1.5×10^{5}	0.1
	50	1.5×10^{9}	1.9×10^{6}	2.3×10^{7}	1.5	7.9×10^{6}	8.2×10^{4}	1.4×10^{4}	0.2
Cherry	1	3.7×10^{8}	2.0×10^{8}	1.5×10^{9}	405.4	9.3×10^{8}	1.5×10^{8}	2.7×10^{8}	29.0
	5	3.0×10^{8}	7.0×10^{8}	1.5×10^{9}	500.0	4.4×10^{8}	1.9×10^{8}	2.2×10^{8}	50.0
	10	8.1×10^{7}	2.3×10^{7}	1.5×10^{8}	185.2	5.4×10^{7}	4.6×10^{6}	2.0×10^{7}	37.0
	20	3.7×10^{6}	9.5×10^{3}	1.8×10^{5}	4.9	4.8×10^{6}	8.6×10^{3}	9.9×10^{3}	0.2
	30	1.3×10^{6}	7.6×10^{4}	1.8×10^{4}	1.4	6.2×10^{5}	9.8×10^{4}	1.5×10^{4}	2.4
	40	6.1×10^{5}	1.5×10^{4}	4.6×10^{2}	0.07	1.0×10^{5}	3.7×10^{4}	1.7×10^{4}	17.0
	50	3.7×10^{4}	1.3×10^{4}	9.9×10^{2}	2.7	2.0×10^{5}	1.0×10^{4}	9.5×10^{3}	4.8
Hawthorn	1	5.0×10^{8}	3.8×10^{8}	5.1×10^{8}	102.0	3.0×10^{8}	1.9×10^{8}	2.5×10^{8}	83.3
	5	4.3×10^{8}	4.2×10^{8}	3.9×10^{8}	90.7	3.0×10^{8}	5.6×10^{7}	1.5×10^{8}	50.0
	10	5.0×10^{8}	1.6×10^{8}	2.2×10^{8}	44.0	3.8×10^{8}	1.8×10^{8}	6.8×10^{7}	17.9
	20	1.6×10^{8}	3.7×10^{7}	9.6×10^{7}	60.0	2.1×10^{8}	1.8×10^{7}	5.1×10^{7}	24.3
	30	3.6×10^{8}	4.8×10^{8}	3.2×10^{8}	88.9	1.0×10^{8}	5.7×10^{6}	3.3×10^{7}	33.0
	40	3.7×10^{8}	3.5×10^{8}	2.3×10^{8}	62.2	2.2×10^{8}	7.6×10^{5}	2.3×10^{5}	0.1
	50	3.4×10^{8}	7.4×10^{7}	9.1×10^{7}	26.8	2.3×10^{8}	3.1×10^{5}	2.9×10^{4}	0.01
Dog Rose	1	7.0×10^{8}	2.8×10^{8}	3.9×10^{8}	55.7	4.4×10^{8}	3.1×10^{8}	2.2×10^{9}	500.0
	5	5.8×10^{8}	$2,5\times10^{8}$	1.4×10^{9}	241.4	8.0×10^{8}	3.3×10^{8}	2.1×10^{9}	262.5
	10	6.4×10^{8}	2.6×10^{8}	2.6×10^{9}	406.3	2.3×10^{8}	1.9×10^{8}	2.2×10^{8}	95.7

	20	1.7×10^{8}	1.0×10^{5}	3.3×10^{4}	0.02	2.6×10^{8}	4.7×10^{7}	1.1×10^{8}	42.3
	30	4.5×10^{7}	2.0×10^{4}	1.2×10^{3}	0.003	1.2×10^{8}	3.2×10^{6}	9.0×10^{6}	7.5
	40	1.2×10^{8}	3.0×10^{5}	1.8×10^{4}	0.02	8.4×10^{7}	6.4×10^{5}	5.3×10^{6}	8.3
	50	7.6×10^{6}	9.9×10^{3}	1.5×10^{3}	0.02	1.9×10^{7}	4.8×10^{5}	8.8×10^{5}	4.6
Gooseberry	1	1.5×10^{9}	3.2×10^{8}	1.3×10^{8}	8.7	1.9×10^{8}	1.8×10^{7}	3.5×10^{7}	18.4
	5	9.5×10^{8}	2.1×10^{8}	7.9×10^{7}	8.3	4.9×10^{7}	3.4×10^{6}	3.8×10^{4}	0.08
	10	9.6×10^{8}	1.8×10^{8}	1.2×10^{8}	12.5	2.5×10^{7}	1.5×10^{5}	1.9×10^{4}	0.08
	20	5.5×10^{8}	2.1×10^{7}	3.4×10^{7}	6.2	2.2×10^{6}	2.5×10^{4}	3.0×10^{3}	0.1
	30	1.4×10^{7}	3.8×10^{3}	3.7×10^{2}	0.003	2.2×10^{6}	2.5×10^{4}	8.5×10^{2}	0.04
	40	2.5×10^{5}	1.4×10^{3}	4.9×10^{2}	0.2	2.9×10^{6}	2.2×10^{3}	1.4×10^{3}	0.05
	50	1.0×10^{5}	2.5×10^{2}	5.5×10^{1}	0.06	2.8×10^{6}	7.5×10^{2}	8.3×10^{2}	0.03
Chokeberry	1	6.5×10^{8}	1.6×10^{9}	1.3×10^{9}	200.0	2.6×10^{8}	9.1×10^{7}	1.8×10^{8}	69.2
	5	2.4×10^{8}	1.7×10^{8}	8.6×10^{8}	358.3	7.3×10^{8}	1.8×10^{8}	1.1×10^{9}	150.7
	10	2.6×10^{8}	1.5×10^{8}	3.4×10^{8}	130.8	7.5×10^{8}	3.1×10^{8}	1.8×10^{9}	240.0
	20	2.8×10^{8}	1.1×10^{7}	1.3×10^{7}	4.6	1.5×10^{6}	3.5×10^{4}	2.1×10^{3}	0.14
	30	7.3×10^{7}	1.7×10^{4}	1.3×10^{6}	1.8	5.0×10^{5}	2.1×10^{4}	2.9×10^{3}	0.6
	40	2.4×10^{6}	1.8×10^{3}	3.4×10^{2}	0.01	1.7×10^{4}	2.2×10^{3}	4.9×10^{2}	2.9
	50	6.9×10^{5}	1.3×10^{4}	1.7×10^{3}	0.2	8.8×10^{4}	4.3×10^{3}	1.7×10^{3}	1.9
Quince	1	3.6×10^{8}	7.9×10^{7}	2.1×10^{8}	58.3	1.9×10^{8}	6.9×10^{7}	2.1×10^{8}	110.5
	5	5.2×10^{8}	4.5×10^{8}	1.9×10^{8}	36.5	1.8×10^{8}	2.3×10^{7}	1.6×10^{8}	88.9
	10	3.7×10^{8}	7.7×10^{7}	5.0×10^{7}	13.5	1.6×10^{8}	1.6×10^{5}	1.9×10^{5}	0.1
	20	1.4×10^{8}	2.8×10^{7}	1.2×10^{7}	8.6	1.3×10^{7}	1.9×10^{4}	1.2×10^{3}	0.009
	30	3.2×10^{7}	8.8×10^{3}	6.1×10^{3}	0.02	5.4×10^{6}	2.4×10^{5}	2.8×10^{4}	0.5
	40	1.7×10^{6}	1.5×10^{2}	8.5×10^{1}	0.005	7.0×10^{6}	3.5×10^{3}	1.4×10^{3}	0.02
	50	1.1×10^{5}	1.3×10^{2}	5.0×10^{1}	0.05	1.2×10^{6}	1.3×10^{3}	2.6×10^{2}	0.02
Japanese quince	1	9.6×10^{7}	1.2×10^{7}	5.8×10^{7}	60.4	1.5×10^{8}	6.5×10^{7}	2.2×10^{8}	146.7
	5				0.02				50.0

		1.1×10^{8}	7.5×10^{4}	2.6×10^{4}		5.0×10^{7}	1.3×10^{7}	2.5×10^{7}	
	10	nt	nt	nt	nt	2.3×10^{7}	6.0×10^{6}	1.7×10^{6}	7.4
	20	nt	nt	nt	nt	nt	nt	nt	nt
	30	nt	nt	nt	nt	nt	nt	nt	nt
	40	nt	nt	nt	nt	nt	nt	nt	nt
	50	nt	nt	nt	nt	nt	nt	nt	nt
control		**4.3×10^{8}**	**2.6×10^{8}**	**5.7×10^{8}**	**132.6**				

nt – not tested

Furthermore, we noticed that both methanol and water chokeberry and dog rose extracts as well as cherry ME constituted a group of fruit extracts characterized by similar efficacy of action. For all these extracts at concentrations from 1 to 10 mg mL^{-1}, *E. coli* 192 strain proliferated in NHS very intensively and the survival of these rods after 180 min of incubation in serum approached 95.7–500.0% of initial cell number. Higher concentrations of plant extracts from this group altered the susceptibility of *E. coli* rods to the lytic activity of NHS in a comparable manner (4.9–2.7% for cherry ME, 4.6 – 0.01 and 2.9 – 0.1% for chokeberry ME and WE, and 0.02-0,003 and 42.3–4.6% for dog rose ME and WE). The least active extract was hawthorn ME, because in all used concentrations the survival ratio of *E. coli* strain after 180 min of incubation in NHS insignificantly decreased and ranged from 102.0 to 26.8% of initial cell number.

Discussion

The lethal effect of human serum on Gram-negative bacteria is well recognized and seems to have an essential role in host defense (Miajlovic and Smith 2014; Berends et al. 2015). This bactericidal activity is mediated by multiple factors, including complement system as a crucial component but also antimicrobial peptides and proteins which enhance lysis of susceptible bacteria by complement. Resistance to complement-mediated serum activity is an important virulence factor of extra-intestinal pathogenic *E. coli* strains isolated from urinary tract or bloodstream infections. The major structures on the surface of *E. coli* rods that confer resistance against the complement cascade are lipopolisaccharride (LPS) and its O-antigen chain, outer membrane proteins (OMP) and the polysaccharide capsules. O-antigens may defend against serum killing by activating complement proteins away from target sites on the bacterial outer membrane or by blocking antibody-binding sites. Consequently, rough strains with LPS which lacks O-antigen side chains are usually more sensitive to serum than smooth strains containing full-length O-chains LPS (Miajlovic and Smith 2014; Johnson 1991). *E. coli* outer membrane protein OmpA has been demonstrated to contribute to serum resistance, increased survival in macrophages and *in vitro* brain microvascular endothelial cells invasion (Weiser and Gotschlich 1991). It was also found that the loss of OmpC increases survival of *E. coli* in human serum by escaping the OmpC-specific antibody-dependent classical complement activation pathway (Liu et al. 2012). Furthermore, expression of the surface-associated polysaccharide capsules (e.g. K1 polysaccharide antigen associated with neonatal meningitis *E. coli*) provides a barrier protecting bacterial outer membrane from deposition of complement factors and Membrane Attack Complex formation (Miajlovic and Smith 2014). Recent studies have also shown that exopolysaccharide colonic acid, traditionally linked with biofilm formation, can be protective against the bactericidal effects of human serum in *E. coli* strains (Miajlovic et al. 2014).

The nature of bacterial resistance to the action of serum has a complex character dependent on the structure and organization of their cell surface. Changes in bacterial membrane stability and permeability through interactions with their surface components may result in loss of serum survival ability. Our present results indicate that the susceptibility of the serum resistant *E. coli* strain to the bactericidal action of normal human serum can be altered by a prior incubation of these rods with methanol and water fruit extracts of raspberry, cherry, hawthorn, dog rose, gooseberry, chokeberry, quince, and Japanese quince. The ability of the studied extracts to affect *E. coli* rods sensitivity to the lytic action of serum was found to be variable and depended on extract concentration. The most noticeable changes in serum resistance of *E. coli* rods were observed in the case of gooseberry (both ME and WE), raspberry ME and cherry WE. The survival ratio after 180 min of incubation in serum of *E. coli* strain, which had previously been incubated for 24 hours in the presence of all tested concentrations (1–50 mg mL^{-1}) of the above-mentioned extracts, ranged from 18.4 to 0.003% of initial cell number. Similar effects for the remaining extracts were recorded only in higher concentrations (20–50 mg mL^{-1}). Methanol hawthorn extract showed the weakest action as *E. coli* strain was sensitive to the lytic activity of serum only at a concentration of 50 mg mL^{-1}. Comparing the influence of studied fruit extracts on susceptibility of *E. coli* rods to bactericidal action of serum with the results of the total polyphenol and anthocyanin content of the extracts (Strugała et al. 2015) it is challenging to indicate accurate correlation. The tested fruit extracts contained ample amounts of polyphenols but differed in composition and anthocyanin amount (hawthorn, dog rose, quince, and Japanese quince extracts possessed almost none or relatively small amounts of anthocyanins/proanthocyanidins; Hendrich et al. 2020). On the other hand, we found that Japanese quince both ME and WE significantly reduced *E. coli* growth and strongly altered the susceptibility of bacteria to the bactericidal action of NHS while hawthorn ME was proven to be the least active extract.

Observed in this report influence of tested extracts on bacterial susceptibility to lytic action of serum may be associated with interactions of plant-derived polyphenolic compounds with components of bacterial cell surface. Such antimicrobial activity was reported for instance by Delehanty and coworkers, who analyzed the binding properties of proanthocyanidins from cranberries, tea and grapes to LPS of such Gram-negative bacteria as *E. coli*, *Salmonella*, *Shigella*, and *Pseudomonas* rods (Delehanty et al. 2007). The authors demonstrated comparable LPS-binding activity for PACs, both those with A- and B-type linkages present in cranberry as well as those with only B-type bonds found in tea and grape. It has been shown also that the recognition of bacterial LPS by PACs appeared to be mediated largely through interaction with the lipid A moiety. Furthermore, PACs specifically inhibited the endocytosis of LPS by blocking its interaction with membrane anchored LPS receptors (TLR4/MD2 and CD14) on human embryonic kidney cells. Likewise, it has been established that plant extracts can interact with protein components of the bacterial cell surface. Liu et al. (2006) showed that some cranberry juice components decreased *E. coli* rods adhesion by direct altering of the P-fimbriae proteins. This interaction induced a shortening of P-fimbriae (via protein compression) and reduced adhesiveness. Decreased expression of outer membrane proteins OmpA, OmpC and OmpF was reported after *E. coli* rods incubation with *Thymus maroccanus* essential oil and its major components (carvacrol and thymol) (Fadli et al. 2014). Altering porin channels (changes in outer membrane proteins OmpC and OmpF expression) leading to susceptibility to β-lactam antibiotics was observed for β-lactam–resistant *Shigella dysenteriae* and *Shigella flexneri* strains, grown in the presence of *Aegle marmelos* water extract (Raja et al. 2008). Furthermore, using proteomic analysis and scanning electron microscope Yong et al. (2015) investigated the antibacterial mechanism of three extracts obtained from antibacterial medicinal plants *Callicarpa formosana*, *Melastoma candidum*, and *Scutellaria barbata* against *E. coli*, *P. aeruginosa*, and *S. aureus* strains. Researchers identified seven differentially expressed bacterial proteins following exposure to tested extracts. Among these proteins, two were associated with bacterial protein translational machinery (30S ribosomal protein S1 and 60 kDa chaperonin), two were linked with bacterial metabolic pathways (triacylglycerol lipase and stringent starvation protein A), and three were involved in integrity of bacterial membranes (N-acetylmuramoyl-l-alanine amidase, and two structural proteins flagellin and OmpA). Moreover,

the morphological changes in *S. aureus* and *E. coli* strains, upon treatment with antibacterial *M. candidum* extract were observed. The treated bacterial cells did not retain the proper grape-shaped or rod-shaped characteristic, cell surfaces were uneven, and the cells appeared damaged.

Even though many studies have shown that plant extracts rich in phenolic compounds such as flavonoids, phenolic acids, lignans and polymeric tannins have antimicrobial activity, the multiple mechanisms of their action and synergies of their components has not been thoroughly investigated up to now (Nohynek et al. 2006). Extensive evaluation of antimicrobial action of plant extracts allows finding alternative mechanisms to prevent infections and look for new plant sources of anti-infective drugs prescribed by physicians. This has become particularly important in recent decades in view of increasing overprescription and misuse of established antibiotics, limited effective life span of drugs derived from microbes, detection of novel pathogens, as well as remarkable abilities of microorganisms to develop resistance against antibiotics (Cowan 1999).

In conclusion, the present study demonstrated that the resistance of *Escherichia coli* rods to the lytic action of normal human serum is altered by a previous incubation of these bacteria with raspberry, cherry, hawthorn, dog rose, gooseberry, chokeberry, quince, and Japanese quince methanol and water extracts. The ability of the studied extracts to affect *E. coli* rods sensitivity to the bactericidal action of serum was found to be variable and depended on extract concentration. The most visible changes in serum susceptibility of *E. coli* rods were observed in the case of both methanol and water gooseberry extracts, as well as raspberry methanol and cherry water extracts. Observed in this report impact of tested extracts on microbial resistance to serum activity may be associated with interactions of plant-derived polyphenolic compounds with components of bacterial cell surface. Evaluation of the strict antimicrobial mechanisms of action of phenolics-rich plant extracts can contribute to define new alternative compounds with potential applications in food industry and biopharmaceutical or medical approaches to preventing and potentially treating pathogenic infections.

Abbreviations

ME methanol extract

WE water extract

NHS normal human serum

Declarations

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DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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