# Microbiological, antioxidant and lipoxygenase-1 inhibitory activities of fruit extracts of chosen *Rosaceae* family species

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#### **Conflict of interest**

None declared

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#### **Abstract**

**Background.** Extracts from the *Rosaceae* family fruits are rich in natural, biologically active polyphenols, but their antibacterial properties are still poorly understood. Therefore, we focused our research on their activity against uropathogenic *Escherichia coli* strains. This research also concerned the proof of their ability to reduce oxidative stress and modulate the activity of lipoxygenase-1 (LOX-1). It is well-known that plants represent a source of bioactive compounds whose antioxidant activity may be useful in protecting against oxidative damage in cells, which have been linked to the pathogenesis of many oxidative diseases.

**Objectives.** The study determined the biological activity of methanol (ME) and water (WE) extracts rich in polyphenols from the hawthorn (*Crataegus monogyna* Jacq.), dog rose (*Rosa canina* L.), quince (*Cydonia oblonga* Mill.), and Japanese quince (*Chaenomeles speciosa* (Sweet) Nakai).

Material and methods. The antioxidant capacity was evaluated using 1,1diphenyl-2-picrylhydrazyl (DPPH\*) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS\*\*) radical scavenging methods. The inhibition of liposome membrane oxidation was studied using the thiobarbituric acid reactive substances assay. Lipoxygenase-1 inhibitory activity was measured using the spectrophotometric method. Bacterial growth was determined by evaluating the number of colony forming units per milliliter (CFU/mL). Hydrophobicity was established with salt aggregation hydrophobicity test (SAT). Swimming and swarming motilities were evaluated using soft-agar plates. Production of curli fimbriae was estimated on CFA agar. The P fimbriae were detected using the hemagglutination of erythrocytes. Adhesion of bacteria to human uroepithelial cells was assessed. The amount of biofilm was determined spectrophotometrically.

**Results.** We showed that most of these extracts are effective antioxidants and free radical scavengers, possess reasonable potential anti-inflammatory activity, reduce the adhesion of *E. coli* to uroepithelial cells, and reduce the ability of these bacteria to form biofilm.

**Conclusions.** The extracts examined, showing very promising biological properties, seem to be able to join the list of substances that can be used as dietary supplements aimed at preventing, for example, urinary tract infections, or as support of drug treatment in many diseases.

Key words: Rosaceae fruit extracts, antimicrobial, antioxidant, lipoxygenase-1 inhibition

## Introduction

In recent years, dozens of scientific articles containing the keywords "cranberry" and "proanthocyanidins" have been published.1 This is a consequence of the commonly known fact that many berries (not only cranberries) contain relatively large amounts of anthocyanins, ellagitannins and proanthocyanidins.<sup>2,3</sup> This, combined with the other well-accepted facts that cranberry juice or extracts possess antibacterial properties, especially that they can reduce the risk of urinary tract infections, might result in the conclusion that the presence of anthocyanins and/or proanthocyanidins in certain plants is essential for their biological and/or antioxidant activity.4,5 To test if this conclusion is true, we have chosen for the present study the methanol (ME) and water (WE) extracts of 4 fruits of plants belonging to the Rosaceae family and (according to the preliminary chemical analysis) possessing almost none or relatively small amounts of anthocyanins/proanthocyanidins (when compared to other bioactive phenolics). The Rosaceae family was chosen because many its members have been used in folk medicine. For example, rose hips (in particular those of the dog rose) were traditionally used as anti-infectious and anti-inflammatory agents.<sup>6</sup> Several North American First Nations have used hawthorn as a remedy against gastrointestinal disorders and respiratory problems like coughs, flu, bronchitis, and asthma, while in Chinese traditional medicine, it was used against circulatory problems, indigestion, diarrhea, and hypertension.<sup>7</sup> On the other hand, quince was used in Italy in folk medicine for treatment of various skin diseases and in Portugal the sedative, antipyretic, antidiarrheal, and antitussive properties of quince leaves were utilized.8 In the current paper, the chemical analysis of the composition of the extract has been followed by an assessment of their antioxidant and lipoxygenase-1 (LOX-1) inhibitory (as a potential anti-inflammatory) properties and antibacterial effects exerted against Escherichia coli rods.

## Material and methods

#### Plants and extract preparation

The raw material to study was hawthorn, dog rose, quince, and Japanese quince (J. quince). Fruits of hawthorn, dog rose and J. quince were collected in Szczytnicki Garden in Wrocław, Poland, whereas the fruits of quince were collected in the Arboretum and Institute of Physiography in Bolestraszyce, Poland. The WE and ME were prepared exactly as previously described by Sroka et al.<sup>9</sup>

## Determination of total phenol content and identification of components

Total polyphenols were determined using the Folin–Ciocalteu method. The results were calculated as the equivalent of gallic acid (in micromoles) per gram of dry matter of the extract ( $\mu$ M GAE/g d.m.).

## Preparation of liposomes and induction of lipid peroxidation

The method of lipid peroxidation assessment was described by Strugała et al. <sup>10</sup> The lipid peroxidation level in the liposomes was measured as the concentration of a thiobarbituric acid reactive substance (TBARS). The antioxidant activity of the extracts tested was expressed with the parameters:  $IC_{50}^{PC}$  (Inhibition Concentration) [mg/mL] and TEAA (Trolox equivalent antioxidant activity expressed in micrograms of Trolox per gram of dry matter of the extract –  $\mu$ M TE/g d.m.).  $IC_{50}^{PC}$  represented the amount of an antioxidant which causes 50% inhibition of phosphatidylcholine (PC) liposome peroxidation.

### Free-radical scavenging assay

The free-radical scavenging activity of 1,1diphenyl-2-pic-rylhydrazyl (DPPH\*) was measured according to the method described by us in an earlier work. The free-radical scavenging activity of cation-radicals (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS\*\*)) was assessed according to the method described by Re et al. The results are expressed as  $\mu M$  TE/g d.m. by reference to a standard curve.

#### **Evaluation of LOX-1 inhibition**

The inhibition of soybean lipoxygenase (Sigma-Aldrich, St. Louis, USA) by fruit extracts was tested using the procedure described by Axelrod et al. 12 with modifications. The WE and ME of the plants at a concentration of 150  $\mu g/mL$ were used. For comparative purposes, the non-steroidal anti-inflammatory agent Ibuprofen was used as a reference substance at a concentration of 0.75 µg/mL. Reactions were carried out in 10 mm path-length quartz cuvettes containing, in a final volume of 2.6 mL: borate buffer pH 9.0, LOX-1 (0.1 mg/mL), the extracts tested, and 50  $\mu$ M linoleic acid. This mixture was incubated for 3 min at room temperature, prior to the measurement. Reference cuvettes (of 2.6 mL volume) contained sodium linoleate borate buffer and an appropriate volume of the solvent extract tested. Inhibition of LOX-1 activity was assessed through spectrophotometric monitoring of the absorbance increase at 234 nm (1 min, 2 min and 3 min after linoleic acid addition) due to formation of conjugated diene hydroperoxides during the enzymatic oxidative processes. Percentage inhibitory effect was calculated using the following formula:

$$\% inhibition = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \cdot 100\%$$

where:  $\Delta A_{control}$  and  $\Delta A_{sample}$  denote the increase of absorbance after 3 min from substrate addition to the probe without or with the extract tested, respectively.

## **Bacterial strain and growth conditions**

A clinical *E. coli* strain isolated from the urine of a patient hospitalized in the University Hospital in Wrocław was used. The *E. coli* stock culture was kept in the refrigerator (–40°C) on a nutrient agar plate. Before each experiment, the strain was first allowed to reach room temperature, transferred to tryptic soy agar, and incubated at 37°C for 18 h. Then, bacterial cells were incubated in tryptic soy broth with varying concentrations (1, 5, 10, 20, 30, 40, 50, 60, 65, 70, 75, and 80 mg/mL) of ME or WE of the plants tested for 24 h at 37°C. Control cultures were incubated in the absence of plant extracts.

### **Antimicrobial activity**

The antibacterial activities of the tested fruit ME and WE were determined with the standard dilution method. After incubation with different concentrations of fruit ME and WE, the bacterial cells were centrifuged, washed 3 times in phosphate buffered saline (PBS), diluted, and cultured on nutrient agar plates for 18 h at 37°C. The effect of the tested extracts on bacterial growth was evaluated on the basis of the number of colony forming units per milliliter (CFU/mL).

## Swimming and swarming motility

Swimming and swarming motilities were evaluated using soft-agar plates according to the procedures described by Hidalgo et al.  $^{13}$  and Sanchez-Torres et al.  $^{14}$ 

## Hydrophobicity of bacterial cells

Bacterial cell hydrophobicity was assessed with the salt aggregation hydrophobicity test (SAT) as described by Siegfried et al.<sup>15</sup>

## The ability to produce curli and P fimbriae

Production of curli fimbriae was estimated by bacterial growth on CFA agar supplemented with Congo red dye as described by Rosser et al. <sup>16</sup> Escherichia coli colonies expressing curli fimbriae are able to bind Congo red dye, so they demonstrate the red color. The P fimbriae expression was detected using the hemagglutination of 3% human erythrocytes (blood group 0) in the presence and absence of 3% (w/v) D-mannose (mannose-resistant hemagglutination), as described by Latham et al. <sup>17</sup>

## Adhesion of bacteria to human uroepithelial cells

The cell adhesion assay was performed essentially as described previously. 18

### **Biofilm formation assay**

The biofilm formation assay was performed according to O'Toole and Kolter. <sup>19</sup> After 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days of incubation, the  $OD_{590}$  values of the stained biofilms were read using a microtiter plate reader. On the basis of the recorded optical densities (ODs) of bacterial biofilms, *E. coli* strains were divided into 4 categories:  $OD \leq ODc$  (no biofilm producer);  $ODc < OD \leq 2 \times ODc$  (weak biofilm producer);  $2 \times ODc < OD \leq 4 \times ODc$  (moderate biofilm producer); and  $4 \times ODc < OD$  (strong biofilm producer). <sup>20</sup> The cut-off OD (ODc) was defined as 4 standard deviations (SD) above the mean OD of the negative control. In our study, the ODc value was 0.003.

### Statistical analysis

The results of all the experiments are given as a mean value ±SD of 3 independent experiments. The data concerning the antioxidant and anti-inflammatory properties was compared using Duncan's multiple range test and analyzed using one-way analysis of variance (ANOVA). The differences in swimming and swarming motilities, bacterial adhesion, and biofilm formation by bacteria exposed and unexposed to fruit extracts were analyzed with a parametric t-test for independent samples using STATISTICA v. 9.0 (StatSoft, Inc., Tulsa, USA). P-values <0.05 were considered to be statistically significant.

#### Results

## Total polyphenol content and extract composition analysis

The results of the total polyphenol content analysis using the Folin–Ciocalteu method of the studied extracts are presented in Table 1. Qualitative and quantitative analysis of the components present in the extracts was performed with the high-performance liquid chromatography (HPLC), and the results are presented in Table 1. As can be seen, individual extracts differed in their composition depending on the method of extraction as well as on the type of fruit. The biggest similarities can be found in the case of flavanols and flavonols that are present in ME of dog rose, quince and J. quince. All these preparations are relatively rich in quercetin 3-O-galactoside and quercetin 3-O-glucoside. These 2 components were also found in hawthorn ME, however, in much smaller amounts than in other extracts. On the other hand, among phenolic

Fruit	TPC [μM GAE/g d.m.]		Flavanols a	nd flavonols	Phenolic acids and derivatives		
	ME	WE	ME	WE	ME	WE	
Hawthorn	58.8±1.9°	23.8±0.7 <sup>d</sup>	(–)epicat (75.0%) f-3-ol dimer (12.0%) f-3-ol trimer (5.7%) q-3-gal (3.8%) q-3-glc (1.2%) q-3-rut (0.3%) n.i. (2%)	n.d.	chlor ac (45.9%) p-coum ac (21.1%) phenolic ac n.i. (13.7%) neochlor ac (8.9%) n.i. (10.4%)	neochlor ac (69.4%) p-coum ac (18.0%) phenolic ac n.i. (8.0%) n.i. (5.0%)	
Dog rose	46.4±2.9 <sup>d</sup>	49.3 ±1.5 <sup>a</sup>	q-3-gal (35.2%) q-3-glc (64.8%)	(+)cat (86.7%) procyanidin n.i. (13.3%)	gall ac (45.3%) ell ac (28.5%) der gall ac (26.2%)	der gall ac (100.0%)	
Quince	152.9 ±4.1 <sup>b</sup>	37.0 ±1.2 <sup>b</sup>	q-3-gal (57.8%) q-3-glc (15.8%) q-3-pento (15.8%) q-3-rut (9.1%) n.i. (1.5%)	q-3-gal (87.5%) q-3-rut (12.5%)	chlor ac (39.8%) phenolic ac n.i. (29.9%) n.i. (30.3%)	chlor ac (38.0%) phenolic ac n.i. (43.9%) n.i. (18.1%)	
Japanese quince	216.5 ±7.2°	30.9 ±1.5°	q-3-gal (54.7%) q-3-glc (18.8%) n.i. (26.5%)	n.d.	chlor ac (40.1%) phenolic ac n.i. (39%) neochlor ac (14.9%)	chlor ac (81.4%) n.i. (19.6%)	

Table 1. Total polyphenol content (TPC) and main groups of phenolic compounds (flavanols and flavonols, phenolic acids, and their derivatives) of methanol (ME) and water (WE) extracts of plants studied

d.m. - dry matter; GAE/g d.m. - equivalent of gallic acid (in micromoles) per gram of dry matter of the extract; (-)epicat - (-)epicatechin; (+)cat - (+)catechin; chlor ac - chlorogenic acid; der gall ac - derivatives of gallic acid; dicaffe ac - dicaffeoylquinic acid; ellq ac - ellagic acid; f-3-ol dimer - flavan-3-ol dimer; f-3-ol trimer – flavan-3-ol trimer; gall ac – gallic acid; neochlor ac – neochlorogenic acid; p-coum ac – p-coumaroquinic acid; phenolic ac – phenolic acid; q-3-gal – quercetin 3-O-galactoside; q-3-glc – quercetin 3-O-glucoside; q-3-rut – quercetin 3-O-rutinoside; q-3-pento – quercetin 3-O-pentoside; n.i. – not identified; n.d. – not determined. Different uppercase letters (a–d) within the same columns indicate significant differences at p < 0.05 in Duncan's test.

n.i. (26.5%)

acids, the common component for hawthorn, quince and J. quince ME, and quince and J. quince WE was chlorogenic acid, which in the case of the last extract mentioned reached the level of 81.4% contribution.

## Free-radical scavenging and antioxidant properties

The results of experiments in which the antioxidant and radical scavenging properties of the extracts studied have been determined are presented in Table 2. As can be seen, both extracts of J. quince appeared to possess the strongest antioxidant properties (as determined with all tests used), and the ME of these fruits was also the most effective free radical scavenger (in both DPPH\* and ABTS\*+ tests). The scavenger activity of all extracts studied is comparable to that measured by Grace et al.<sup>21</sup> for cranberries; however, one has to keep in mind that the activities mentioned were calculated with respect to the fresh weight of the berries (not dry mass as in our case). The weakest antioxidant properties were recorded for both types of quince extracts whereas dog rose extracts were found to be the poorest free radical scavengers.

dicaffe ac (4.6%)

#### Effects of the extracts on LOX-1 inhibition

As it follows from Table 3, all extracts studied were able to inhibit soybean LOX to an extent comparable to the inhibition effects exerted by the 200-fold smaller

Table 2. Antioxidant ( $IC_{50}^{PC}$ ), Trolox Equivalent Antioxidant Activity (TEAA) and antiradical (DPPH', ABTS+) activities of methanol (ME) and water (WE) extracts from fruit of the Rosaceae family

Fruit	IC₅₀ <sup>PC</sup> [mg/mL]		TEAA [μΜ TE/g d.m.]		DPPH <sup>.</sup> [µM TE/g d.m.]		ABTS <sup>+-</sup> [μM TE/g d.m.]	
	ME	WE	ME	WE	ME	WE	ME	WE
Hawthorn	0.148 <sup>b</sup>	1.119 <sup>b</sup>	80.90 <sup>b</sup>	23.80 <sup>c</sup>	234.6 <sup>b</sup>	103.6 <sup>b</sup>	479.6 <sup>b</sup>	186.2 <sup>c</sup>
	±0.002	±0.005	±1.1	±0.04	±9.5	±2.5	±14.5	±2.5
Dog rose	0.160 <sup>b</sup>	0.208 <sup>c</sup>	78.40 <sup>b</sup>	55.70 <sup>b</sup>	171.1 <sup>c</sup>	150.7 <sup>a</sup>	370.7 <sup>d</sup>	365.2 <sup>a</sup>
	±0.035	±0.010	±1.0	±2.70	±5.1	±3.2	±9.6	±12.2
Quince	0.183 <sup>a</sup> ±0.002	1.792 <sup>a</sup> ±0.002	65.40° ±1.10	10.90 <sup>d</sup> ±0.50	232.8 <sup>b</sup> ±2.9	111.5 <sup>b</sup> ±4.4	390.5° ±17.3	247.5 <sup>b</sup> ±3.7
Japanese	0.124 <sup>c</sup>	0.089 <sup>d</sup>	96.76 <sup>a</sup>	134.6 <sup>a</sup>	275.3°	73.4°	547.7°	162.4 <sup>d</sup>
quince	±0.040	±0.042	±3.10	±10.60	±4.4	±1.4	±35.4	±2.0

Different uppercase letters (a–d) within the same columns indicate significant differences at p < 0.05 in Duncan's test.  $IC_{50}^{PC}$  – the concentration of antioxidant which reduces peroxidation intensity of phosphatidylcholine liposomes by about 50%.

**Table 3.** Lipoxygenase (LOX-1) inhibitory activity (%) of methanol (ME) and water (WE) extracts at 150  $\mu$ g/mL and non-steroidal anti-inflammatory agent lbuprofen at 0.75  $\mu$ g/mL

Examined	% LOX-1 inhibition				
substance	ME	WE			
Hawthorn	22.6 ±3.4°	47.7 ±1.5ª			
Dog rose	45.7 ±1.0°	42.8 ±1.5 <sup>b</sup>			
Quince	10.3 ±2.3 <sup>d</sup> ↑	31.9 ±2.4°			
Japanese quince	39.8 ±1.8 <sup>b</sup>	25.9 ±0.5 <sup>d</sup>			
Ibuprofen	26.1	±1.6			

<sup>1</sup> – increase of LOX-1 activity. Different uppercase letters (a–d) within the same columns indicate significant differences at p < 0.05 in Duncan's test.

concentration of Ibuprofen. The most effective inhibitors were dog rose ME and WE and hawthorn WE; however, other extracts (with the striking exception of quince ME) also showed reasonable levels of LOX-1 inhibition. Somewhat surprisingly, we found that quince ME exerted the opposite effect to that of other extracts and increased the LOX-1 activity by approx. 10%.

### Effects of the extracts on bacterial growth

Very low concentrations of hawthorn, dog rose, quince, and J. quince ME and WE had no effect on the survival of the bacteria (Table 4). In all cases, the values of CFU/mL were comparable to controls. In this group of extracts, the best bactericidal activity was observed for J. quince (both ME and WE). The values of CFU/mL at concentrations from 20 mg/mL to 80 mg/mL were gradually decreased to approx.  $10^4$ – $10^5$  bacteria per milliliter. A much weaker effect was found for extracts of dog rose and quince. Both ME and WE at concentrations ranging

from 20 mg/mL to 80 mg/mL slightly reduced the number of bacterial cells compared to the control. Both WE and ME of hawthorn had no impact on the survival of bacteria. For all concentrations of ME and WE used in our study, the values of CFU/mL were comparable to the control.

## Effects of the extracts on *E. coli* swimming and swarming motility

An analysis of the average motility zone diameters of *E. coli* incubated with the extracts studied shows that no effects on the E. coli swimming ability were exerted by either ME or WE of dog rose or quince, nor by hawthorn ME (Fig. 1). The average swimming motility zone diameters of *E. coli* rods after incubation of the cells with various concentrations of these extracts were slightly lower or comparable to the control (30.0  $\pm$ 2.0 mm). Water extract of hawthorn in concentrations ranging from 40 mg/mL to 80 mg/mL reduced the swimming motility zone diameters of *E. coli* down to the value of 10.0 ±1.0 mm, while in the case of lower concentrations of this extract, no such effect was found. The average swimming motility zone diameters of E. coli rods after incubation of the cells with various concentrations of J. quince were lower than the control  $(21.0 \pm 2.0 \text{ mm} \text{ and } 17.0 \pm 1.0 \text{ mm} \text{ for ME and WE, respec-}$ tively). The swarming ability of the examined rods was strongly affected by almost all concentrations of hawthorn (both ME and WE) and dog rose ME (Fig. 2). In those cases, the average swarming zone diameter was reduced from  $16.0 \pm 2.0$  mm (control) to 7–8 mm. Such effect was also noted for higher concentrations of quince and J. quince ME, where the observed swarming motility zone diameters decreased to 6–7 mm. The average swarming motility zone diameters of *E. coli* rods after incubation of the cells with

 $\textbf{Table 4.} \ \textit{Effect of methanol (ME)} \ \textit{and water (WE) extracts of hawthorn, dog rose, quince, and J. quince on \textit{E. coli} \ \textit{growth} \ \textit{and proved to the provided of the provided o$ 

Plant extract	Viable cell counts [CFU/mL] [×10°]							
concentration	hawthorn		dog rose		quince		Japanese quince	
[mg/mL]	ME	WE	ME	WE	ME	WE	ME	WE
Control	2.4							
1	_a	-	=	-	=	-	3.2	2.2
5	-	-	_	-	-	-	2.2	1.9
10	2.5	2.3	1.3	1.2	1.6	1.2	-	-
20	1.7	1.8	1.1	0.67	0.68	1.2	0.089	0.027
30	1.6	1.8	1.0	0.67	0.66	0.73	0.086	0.0076
40	1.6	1.7	0.76	0.34	0.59	0.70	0.0023	0.0044
50	1.5	1.6	0.62	0.2	0.57	0.52	0.00021	0.0012
60	1.5	1.5	0.51	0.078	0.51	0.50	0.00021	0.00068
65	1.4	1.2	0.47	0.073	0.35	0.49	0.000033	0.00048
70	1.4	1.2	0.36	0.066	0.35	0.21	0.000031	0.00032
75	1.3	1.1	0.34	0.026	0.30	0.20	0.000019	-
80	1.3	1.1	0.25	0.020	0.21	0.14	0.000014	-

<sup>&</sup>lt;sup>a</sup> not tested; CFU – colony forming units.

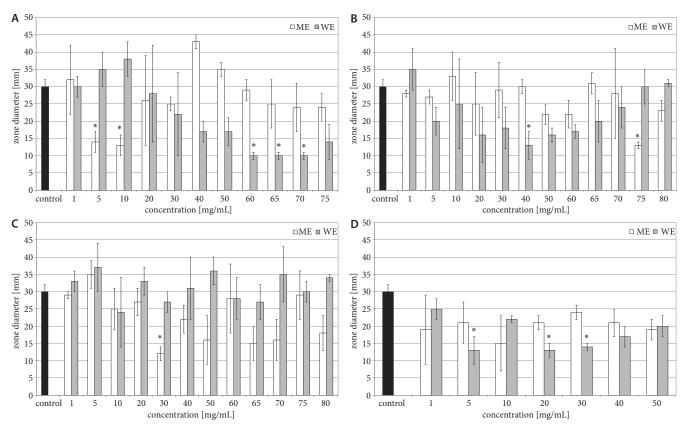


Fig. 1. Effect of methanol (ME) and water (WE) extracts of hawthorn (A), dog rose (B), quince (C), and J. quince (D) on swimming motility of E. coli strain; p < 0.05

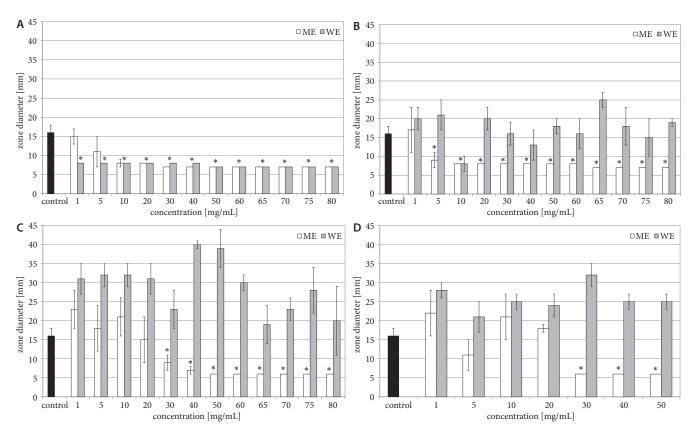


Fig. 2. Effect of methanol (ME) and water (WE) extracts of hawthorn (A), dog rose (B), quince (C), and J. quince (D) on swarming motility of E. coli strain; \*p < 0.05

various concentrations of quince and J. quince WE were higher than in the control (25.0  $\pm$ 2.0 mm). A similar effect on the swarming ability of the *E. coli* rods examined was also shown by dog rose WE. The average swarming motility zone diameters were slightly higher or comparable to the control (20.0  $\pm$ 2.0 mm).

## Effects of the extracts on the hydrophobicity of bacterial cells and on the ability to produce curli and P fimbriae

We observed that none of the tested plant extracts affected the hydrophobic properties of the bacterial cell surface. We also noted that the ability of *E. coli* to produce curli and P fimbriae was not altered by incubation of these bacteria with all tested plant extracts.

## Effects of the extracts on the adhesion of bacteria to human uroepithelial cells

The dependencies of the percentage of adhesion of bacterial cells on fruit extract concentration are shown in Fig. 3 for hawthorn, dog rose, quince, and J. quince, respectively. This figure shows that there were almost no differences in the efficacy of ME and WE; however, certain fruit extracts differed in their activity. Out of the 4 extracts studied herein, only hawthorn extract showed poor effects

and weakly reduced adhesion of bacteria to epithelial cells (Fig. 3A), even at high concentrations (70–80 mg/mL). Much stronger effects were recorded for dog rose and quince extracts (Fig. 3B), which were able to reduce percentage of adhesion to less than 10% when used at high concentrations. The strongest effects were found for J. quince (Fig. 3D), for which a significant decrease of adhesion was recorded for concentrations exceeding 20 mg/mL.

## Effects of the extract on biofilm formation

The control samples of *E. coli* incubated up to 10 days in the absence of the fruit extracts studied herein have shown that the amounts of biofilm formed by these bacteria changed in time, reaching maxima on approx. the 3rd and 8<sup>th</sup> day of incubation (see the control bars in any panel of Fig. 4). Incubation of the bacteria with the addition of the fruit extracts studied affected the growth of biofilm within the region of the 1st and/or 2nd maximum, depending on the type of extract. The strongest reduction of biofilm was observed when the bacteria were incubated with WE of J. quince (Fig. 4D - note that J. quince was used at half of the concentrations used for other extracts) and quince, for which complete disappearance of the biofilm was observed during the whole time of observation. The ME of J. quince also showed strong biofilm reducing properties; however, the presence of some biofilm remains

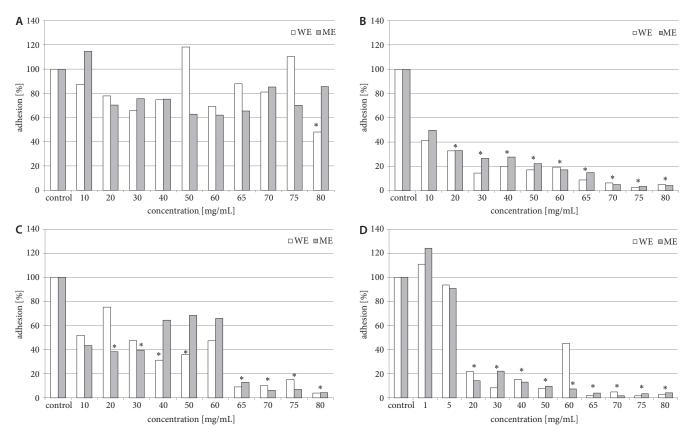
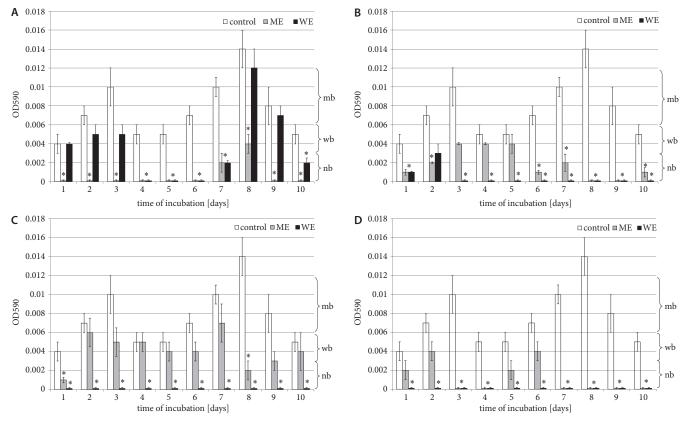


Fig. 3. Effect of water (WE) and methanol (ME) extracts of hawthorn (A), dog rose (B), quince (C), and J. quince (D) on adhesion of *E. coli* to human epithelial cells; \*p < 0.05



**Fig. 4.** Effect of water (WE) and methanol (ME) extracts of hawthorn (A), dog rose (B), quince (C), and J. quince (D) on biofilm formation ability of *E. coli* rods. Abbreviations used for biofilm classification: nb – no biofilm; wb – weak biofilm; mb – moderate biofilm. Bars exceeding mb are classified as strong biofilm; \*p < 0.05.

(classified as a weak biofilm) were recorded on the  $2^{\rm nd}$  and  $6^{\rm th}$  day of incubation. Effects comparable to those described above were also observed for hawthorn ME and dog rose WE (Fig. 4A,B). The weakest effect on the biofilm formation ability was exerted by quince ME (Fig. 3C), and the effects of dog rose ME can also be described as moderate.

#### Discussion

In recent years, much attention has been focused on plants containing various bioactive compounds which may demonstrate some pharmacological properties.<sup>22</sup> In one aspect of our research, we used a model lipid membrane (liposomes that are a spherical bilayer) as a matrix of biological membranes. Membranes are the first target of attack by exogenous free radicals at the cellular level. Peroxidation processes of membrane lipids result in disruption of their function, which is the basis of serious diseases, e.g., atherosclerosis or diabetes. Another important aspect of the research was to check whether natural plant extracts can reduce the risk of urinary tract infections. We determined the phenolic content, antioxidant activity and potential anti-inflammatory properties of 4 plant WE and ME on one hand and assessed the effects exerted by those extracts on *E. coli* on the other hand.

The research presented herein has shown that extracts from the *Rosaceae* family species are rich in phenolic compounds. Quantitative/qualitative identification using HPLC showed that the components of individual extracts belong to 3 groups of phenolic compounds: flavanols, flavonols and phenolic acids. These 3 groups of phenolic compounds in hawthorn extracts, dog rose quince and J. quince were also identified by other researchers.  $^{23-26}$  The richest in phenolic compounds were the ME of J. quince (216.5  $\pm 7.2\,\mu\text{M}$  GAE/g d.m.) and quince (152.9  $\pm 4.1\,\mu\text{M}$  GAE/g d.m.). Chlorogenic acid was identified in all examined extracts.

Comparing the properties of the 2 types of extracts (ME vs WE), one can conclude that in most cases, ME are more effective as antioxidants as well as free radical scavengers. Statistical analysis of the above described data showed that there is no correlation between the total polyphenol content (TPC) and antioxidant properties (measured as  $\rm IC_{50}^{PC}$ ) of the extracts studied (correlation coefficients were –0.338 and –0.273 for ME and WE, respectively). Simultaneously, some correlations were found between the TPC and radical scavenging properties of the extracts: correlation coefficients were 0.817 and 0.782 (in DPPH assay, ME and WE, respectively) and 0.577 and 0.927 (in ABTS assay, ME and WE, respectively). Bearing in mind the above statistical relations and comparing the results of the chemical analysis, it is hard to point to any strict correlation

between the composition of the extracts and their antioxidant/protective action in relation to oxidized lipid membranes. However, we can firmly state that the ME extract of J. quince was at the top of the tables (both phenolic content and antioxidant activity). The antioxidant and antiradical activity of J. quince has been confirmed using different methods in current literature.<sup>27</sup> Until now, to our knowledge, there are no reports related to the inhibition of the peroxidation of lipid systems mimicking the bilayer of biological membranes. Some reviews have been published that contain a wide range of information on both Chaenomeles composition and therapeutic properties. 28,29 It is noteworthy that despite the relatively low but comparable TPC content of WE and ME of the dog rose, they demonstrate a high ability to inhibit lipid peroxidation and LOX-1 activity. Dog rose is also good scavenger against DPPH and ABTS mimic radicals. In mammalian cells, LOX plays a key role in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acid, leukotrienes, lipoxins, and hepoxylines. Antioxidants interact non-specifically with LOX by scavenging radical intermediates and/or reducing the active heme site. 30 For example, in the work of Chen et al., it was shown that extract from Prunus campanulata Maxim, a member of the family Rosaceae, at concentrations of 25 µg/mL, 50 µg/mL and 100 μg/mL causes inhibitory activity of 15-lipoxygenase (15-LOX).31 Tumbas et al. suggest antioxidant properties of wild rose tea (as detected with DPPH' test), presumably related to the content of vitamin C and flavonoid compounds. Simultaneously, the anti-proliferative properties of this tea found in several types of human cancer cells are suggested to result from the interaction with polyphenols.<sup>32</sup> The review of Patel in various aspects sums up the research on the biological activity of fruits of the rose hip, and it indicates the research on the mechanisms of its pharmacological action.<sup>33</sup>

Anthocyanins/proanthocyanidins are often recognized as compounds that play a major role in the antibacterial activities of different fruit juices or extracts. 1,4,34 Despite this, the results presented above demonstrate that many potentially useful properties can also be present in the extracts of fruits that possess a low level or none of such components. Since in our experiments we used the uropathogenic strains of *E. coli*, it seems obvious that as the most important we consider the effects exerted on those bacteria properties which are involved in the induction of urinary tract infection. Swimming and swarming motility and adhesion to the epithelial cells as well as biofilm forming ability undoubtedly belong to these properties.<sup>35</sup> Analysis of the results obtained in our study leads to the conclusion that the most effective was the WE and ME of J. quince, which significantly reduced the swimming and swarming (except for WE) motility, adhesion to epithelial cells and biofilm formation ability of bacteria. Biofilm formation ability was reduced (to different extents) by all extracts studied and adhesion to the epithelial cell was reduced

by all except hawthorn ME and WE. These last extracts have, however, significantly decreased the swarming motility of *E. coli*.

With the exception of hawthorn ME and WE, all extracts also showed some antibacterial properties, however, due to the moderate effect, it was not possible to determine the minimal inhibitory concentration (MIC) in the range of the extract concentrations used in our experiments. The antibacterial activity of these extracts was somehow smaller than that of cranberry extract Żuravit S-O-S®, for which the MIC values were found to be 38 mg/mL or 55 mg/mL, depending on the bacterial strain used in MIC determination. <sup>19</sup> It seems worth emphasizing that apart from antibacterial properties, the fruit extracts studied also showed some anti-inflammatory properties and they appeared to be efficient free radical scavengers and antioxidant agents.

### **Conclusions**

A significant finding in the current work is that it describes the antioxidant, antiradical, anti-inflammatory, and antimicrobial properties of polyphenol-rich extracts. The analyzed plants belonging to the *Rosaceae* family may be considered as valuable agents protecting in vitro the model lipid membrane against peroxidation and can reduce the risk of urinary tract infections. Japanese quince and dog rose extracts in particular, showing very promising biological properties, seem to be able to join the list of substances that can be used as dietary supplements aimed at preventing, for example, urinary tract infections, or as supportive of drug treatment in many diseases.

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