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Role of Structural Changes Induced in Biological Membranes by Hydrolysable Tannins from Sumac Leaves (*Rhus typhina* L.) in their Antihemolytic and Antibacterial Effects

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Abstract In this study, we found that the sumac tannins (*Rhus typhina* L.) exert to a various extent antihemolytic effects and antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* depending on structural specificity of bacteria and different mechanisms of their toxic action. The sumac tannins exert the most expressed activity against *B. cereus.* The antihemolytic effect of the sumac tannins seems to be connected to a greater extent with their modifying action on the erythrocyte membrane structure. It was found that the sumac tannins are incorporated into the erythrocyte membrane, causing transformation of discocytes into echinocytes and enhancing the rigidity of the hydrophilic region of the lipid bilayer. We suggest that the embedding of sumac tannins into the membrane of erythrocytes alters their physical properties and, as a consequence, can limit their interaction with bacterial toxins.

Keywords Tannins · Membrane fluidity · Erythrocyte · Hemolysis · Bacteria

Abbreviations

CytK	Cytotoxin K
DPH	1,6-Diphenyl-1,3,5-hexatriene
GP	Generalized polarization
HBL	Hemolysin BL
LB	Luria–Bertani broth
MIC	Minimal inhibition concentration
NHE	Nonhemolytic enterotoxin
TMA-DPH	1-(4-Trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene
T3SS	Type 3 secretion system

Introduction

Tannins, secondary plant metabolites, are characterized by high chemical activity and a variety of biological effects, such as antitumor, antimutagenic, antimicrobial, antiviral, antiplatelet, hypoglycemic, and anti-inflammatory ones (Koleckar et al. 2008; Serrano et al. 2009). In fact, many biological effects of tannins are due to antioxidant properties, including both antiradical activity and inhibition of the Fenton reaction as a result of metal chelating. The diverse biological activity of tannins is also related to the interaction with biomolecules, and primarily with proteins. Tannins can also regulate the functional activity of cells by

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changing the structure of the membrane due to interaction with lipids (Tarahovsky 2008).

As noted above, tannins exhibit strong antibacterial activity. It is assumed that several mechanisms, such as inhibition of extracellular microbial enzymes, complexation of metal ions, and/or deprivation of substrates, are the basis of this effect (Scalbert 1991; Buzzini et al. 2008). Moreover, tannins can also protect cells against the toxic action of bacteria, directly precipitating endotoxins produced by bacteria or by changes in the properties of membranes resulting in limitation of their incorporation into membrane. Previously it was shown that catechins of tea extract inhibit hemolysis of rabbit erythrocytes induced by staphylococcal α-toxin and *Vibrio parahaemolyticus* hemolysin (Okubo et al. 1989). Moreover, tannins form aggregates with staphylococcal α-toxin and neutralize its toxicity at a cellular and whole-body level in animals (Choi et al. 2007).

Bacillus cereus, a Gram-positive, motile, and aerobic sporeformer, occurring in highly diverse habitats (Swiecicka 2008), may cause nongastrointestinal diseases, e.g., mastitis, meningitis, systemic infection, and respiratory tract infection (Gray et al. 1999; Gaur et al. 2001), as well as emetic intoxication and diarrheal infections (Kotiranta et al. 2000). While emesis is related to cereulide, a small ring-formed dodecadepsipeptide (Agata et al. 1994), three-component enterotoxins, hemolysin BL (HBL), and non-hemolytic enterotoxin (NHE) are responsible for diarrhea (Bartoszewicz et al. 2006; Thaenthanee et al. 2005; Ghelardi et al. 2007). Some *B. cereus* strains produce pore-forming toxins, such as cereolysin, a thiol-activated hemolysin (Kotiranta et al. 2000), hemolysin II (Miles et al. 2002; Andreeva et al. 2007), hemolysin III (Baida and Kuzmin 1996), and cytotoxin K (CytK) (Lund et al. 2000). These bacilli possess also a set of degradative enzymes, e.g., phospholipases and sphingomyelinase, efficiently contributing to food spoilage and the pathogenicity (Kotiranta et al. 2000; Oda et al. 2010).

Pseudomonas aeruginosa, a ubiquitous Gram-negative motile rod, is responsible for acute and chronic infections of the urinary tract, lower respiratory tract, and bones, and also for endocarditis, meningitis, brain abscess, and sepsis (Filiatrault et al. 2006; Mena and Gerba 2009). The pathogenicity of these bacteria is determined by the ability to invade and persist in the tissues, as well as by a set of cytotoxic agents, such as exotoxin A and S, pyocyanin, and phospholipase C, causing hemolysis. Moreover, *P. aeruginosa* secrete toxins with enzymatic activities that span the inner bacterial membrane (Galle et al. 2012).

In this study, mechanism of antihemolytic effects and antibacterial activity of hydrolysable tannins from sumac leaves (*Rhus typhina* L.) against *B. cereus* and *P. aeruginosa* were studied.

Materials and Methods

Plant Material

Leaves of *R. typhina* L. (Anacardiaceae) were collected in the valley of Tashkent (Uzbekistan) and were identified taxonomically by Professor O. P. Pratov (Institute of Botanic, Tashkent, Uzbekistan). Water-soluble extract from the leaves was prepared according to Islambekov et al. (1994).

The extract contains 3,6-bis-*O*-di-*O*-galloyl-1,2,4-tri-*O*-galloyl-β-D-glucose (74.05 %), rutin (1.15 %), 2,3-di-*O*-galloyl-β-D-glucose (2.15 %), 2-*O*-galloyl-β-D-glucose (2.10 %), 3-*O*-galloyl-β-D-glucose (2.20 %), 6-*O*-galloyl-β-D-glucose (2.05 %), 1,4,6-tri-*O*-galloyl-β-D-glucose (5.10 %), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (10.05 %), quercetin (0.05 %), kaempferol (0.05 %), and gallic acid (1.05 %). This extract is water-soluble, has low toxicity (LD_{50} 5,600 mg/kg), and showed interferon-inducing activity (Salikhov et al. 2006).

Bacterial Strains

Bacillus cereus ATCC 10987 (American Type Culture Collection) and *P. aeruginosa* PCM 2270 (Polish Collection of Microorganisms) were used in this study. Depending on the experiment, bacteria grew at 37 °C on nutrient agar plates or in Luria–Bertani (LB) broth with shaking at 250 rpm. The media were obtained from Oxoid (Basingstoke, England).

Bacillus cereus B771 hemolysin II was purified according to Andreeva et al. (2006). The electrophoretical purity of the hemolysin II was equal to 97 %.

Chemicals

6-Dodecanoyl-2-dimethylaminonaphthalene (Laurdan) was from Molecular Probes (Eugene, OR). 1,6-Diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) were from Sigma-Aldrich (St. Louis, MO). All other reagents were purchased from POCH (Poland).

Antibacterial Activity

Bacillus cereus and *P. aeruginosa* were grown overnight in LB broth at 37 °C with shaking at 250 rpm. Then, *B. cereus* was reinoculated into LB broth and grew at the same conditions as above for 4 h. The cells of both bacteria used in this experiment were sedimented by centrifuging at 5,000×*g* for 10 min at 4 °C, washed with sodium saline (0.9 % NaCl), and suspend in sodium saline to optical density of 0.02 and 0.2 for *B. cereus* and *P. aeruginosa*, respectively, measured at 600 nm.

100 µl of bacterial suspensions were inoculated on LB agar and prepared as "lawn plate." Then, disks with 5 mm diameter made of filtration paper were saturated with aqueous solution of the sumac tannins at various concentrations (1–5 mg/ml) and were aseptically put on the surface of the media. Disks soaked with water were used as negative control. Bacteria were incubated overnight at 37 °C. Minimal inhibition concentration (MIC) is considered as the concentration of the preparation in the last disk with the zone of growth inhibition.

Determination of Antihemolytic Activity

Human or swine blood was collected in tubes containing 3.8 % citrate as an anticoagulant (1:9). Citrated blood was centrifuged (2,655×*g*, 15 min, 4 °C), and plasma and buffy coat were removed by aspiration. Erythrocytes were washed twice with 0.9 % NaCl, and then 2 % suspension was prepared. Then, 1 ml of 2 % suspension of erythrocytes was incubated with 1 ml of bacterial suspension prepared as above in the presence or absence of the sumac tannins. After incubation for 30, 60, and 90 min at 38 °C, 0.5 ml of suspension was taken from every sample and mixed with 2 ml of 0.9 % NaCl. For obtaining of 100 % hemolysis, 2 ml of water was added to the control sample. In the case of study of the toxin-induced hemolysis, 0.5 % suspension of human erythrocytes in sodium phosphate buffer pH 6.8 containing NaCl was incubated with hemolysin II (5 ng/ml) with and without the sumac tannins for 30 min. All the samples were centrifuged, and absorbance of supernatants was measured at 540 nm (Sinev et al. 1993). Obtained results are presented as percent of hemolysis depending on concentration of tannins.

Measurement of Erythrocyte Membrane Fluidity

The erythrocyte membrane fluidity was measured by fluorescent probe Laurdan. Suspension of erythrocyte (2 ml of 0.05 % hematocrit in PBS, pH 7.4), after the addition of 1 µM Laurdan, was incubated with the sumac tannins for 15 min at 37 °C. The fluorescence measurements were performed using a Perkin Elmer LS-55 spectrofluorimeter. The excitation wavelength for Laurdan was $\lambda_{ex} = 360$ nm, and the emission was recorded at two wavelengths, $\lambda_{em1} = 440$ nm and $\lambda_{em2} = 490$ nm. Erythrocyte membrane fluidity was determined on the basis of the generalized polarization (GP) of Laurdan, calculated with the formula

$$\mathrm{GP} = \frac{I_1 - I_2}{I_1 + I_2},$$

where I_1 is the fluorescence intensity at $\lambda_{em1} = 440$ nm and I_2 is the fluorescence intensity at $\lambda_{em2} = 490$ nm.

Determination of Erythrocytes Shapes

Suspension of erythrocyte (1 ml of 2 % hematocrit in PBS, pH 7.4) was incubated with the sumac tannins for 15 min at 37 °C. After incubation, samples were fixed with 0.2 % solution of glutaraldehyde. Next, erythrocyte suspension was observed under a microscope Nikon Eclipse E200 equipped with a digital camera. The obtained photographs made it possible to count erythrocytes of various shapes, and then to assess the percent of two basic forms (echinocytes and stomatocytes) in a population of 500 cells (Włoch et al. 2013). The individual forms of erythrocyte cells were ascribed morphological indices according to Bessis scale (1977), which for stomatocytes and echinocytes assume negative and positive values, respectively.

Measurement of Bacterial Cytoplasmic Membrane Fluidity

Bacterial cells were sedimented by centrifuging at 5,000×*g* for 10 min at 4 °C, washed with sodium saline (0.9 % NaCl), and suspend in sodium saline to optical density of 0.02 measured at 600 nm. 1 µM DPH (in tetrahydrofuran) or TMA-DPH (in methanol) was added to 2 ml bacterial suspension and incubated for 20 min in the dark at room temperature. The cuvette holder was temperature controlled by a water thermostat at 37 °C. The readings were taken at intervals of 2 s. The polarization values (r) of the samples were calculated by the fluorescence data manager program using the Jablonski equation:

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}},$$

where I_{VV} and I_{VH} are the vertical and horizontal fluorescence intensities, respectively, of the vertical polarization of the excitation light beam. The factor $G = I_{HV}/I_{HH}$ (grating correction factor) corrects the polarizing effects of the monochromator. The excitation wavelengths were 348 nm (DPH) and 340 nm (TMA-DPH), and the fluorescence emission was measured at 426 nm for DPH and 430 nm for TMA-DPH (Olchowik et al. 2012).

Statistical Analysis

The results are presented as mean ± standard error mean (SEM). The level of significance was analyzed by one-way analysis of variance test (ANOVA) using Origin 8.5.1.

Results and Discussion

The antihemolytic and antimicrobial activity against Gram-negative *P. aeruginosa* and Gram-positive *B. cereus* was

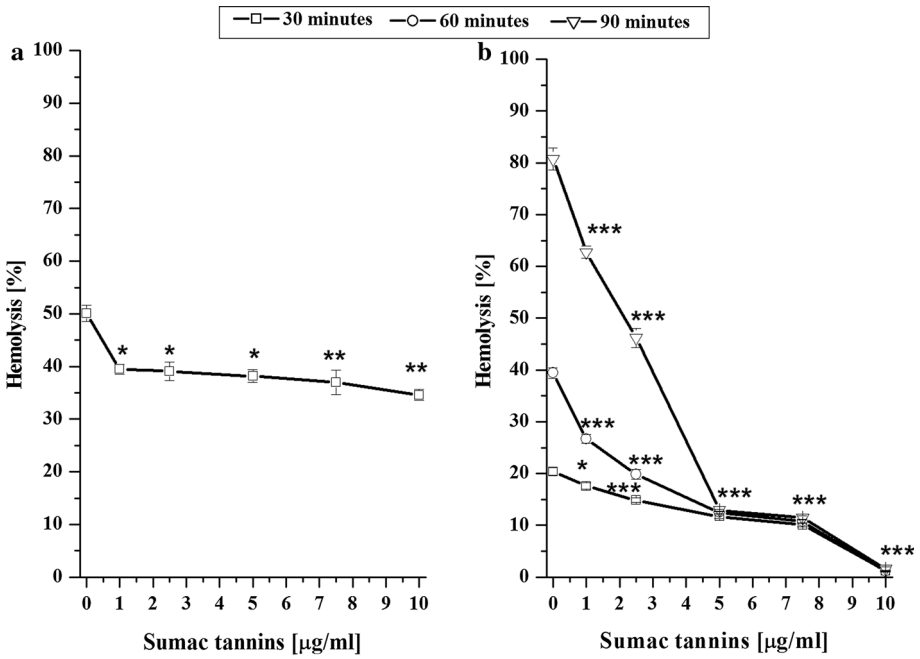

Fig. 1 Protective effects of the sumac tannin against hemolysis induced by incubation of swine erythrocytes with *P. aeruginosa* (**a**) and *B. cereus* (**b**). The data presented are the mean ± SE ($n = 9$). The effect of the sumac tannin was statistically significant according to one-way ANOVA test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Experiment conditions are described in the "Materials and Methods" section

established in the extract from sumac (*R. typhina* L.) leaves, presented by hydrolysable tannins in about 90 %.

Antihemolytic Effects

As depicted in Fig. 1a, incubation of swine erythrocytes with *P. aeruginosa* for 30 min caused 50.08 ± 1.56 % hemolysis. The results indicated also that hemolysis of erythrocytes induced by these bacteria showed a weak dependence on the sumac tannin concentration (Fig. 1a). The sumac tannins at the concentration of 1 μg/ml decreased hemolysis by 10.59 ± 2.22 %, but a further increase of the sumac tannin concentration did not significantly change this parameter. At a tenfold higher concentration of the sumac tannins (10 μg/ml), the percentage of hemolysis inhibition was 15.58 ± 1.12 %. However, in contrast to the above results, we observed strong influence of the sumac tannins on hemolysis of swine erythrocytes caused by *B. cereus* (Fig. 1b). The 30-min incubation of the erythrocytes with these bacteria resulted in hemolysis of 20.40 ± 0.75 % of erythrocytes. In this case, inhibition of hemolysis induced by *B. cereus* by the sumac tannins was of a strong concentration-dependent character in the range 1–10 μg/ml. Prolonging the erythrocyte incubation time with *B. cereus* up to 60 min caused a twofold increase of hemolysis (39.45 ± 1.01 %), and up to 90 min prolongation caused a fourfold increase (85.24 ± 3.27 %), in comparison with the 30-min exposure (20.40 ± 0.75 %). Nevertheless, in both cases, we observed that the sumac tannins at the concentrations of 5, 7.5, and 10 μg/ml displayed almost equal efficiency in protection against hemolysis of erythrocytes caused by *B. cereus*.

The effect of the sumac tannins on the hemolysis of human erythrocytes under the influence of *B. cereus* also was studied (Fig. 2a). Hemolysis of human erythrocytes exposed to *B. cereus* for 60 min in the presence of the sumac tannins at the concentrations of 2.5, 5, 7.5, and 10 μg/ml was reduced to 27.38 ± 0.77, 15.08 ± 0.88, 8.81 ± 1.04 and 1.81 ± 0.29 % in comparison to the effect of only bacteria, which was 35.96 ± 2.63 %. Extension of the incubation of the erythrocytes with *B. cereus* for 90 min resulted in an increase in the lysis of the erythrocytes to 85.24 ± 3.27 %. However, exposure of erythrocytes to sumac tannins for the same period still showed their potent concentration-dependent antihemolytic action (Fig. 2a).

In order to elucidate the mechanism of such high antihemolytic activity of the sumac tannins against *B. cereus* bacteria, we studied their influence on the hemolytic activity of hemolysin II purified from *B. cereus* B771 having a high percentage of homology with CytK, the hemolytic protein of *B. cereus* ATCC 10987 (Lund et al. 2000; Shadrin et al. 2007). As shown in Fig. 2b, the sumac tannins also displayed a protective effect against hemolysis of human erythrocytes caused by hemolysin II. It is worth emphasizing that the concentrations of the sumac tannins of 3 and 5 μg/ml completely inhibited hemolysis caused by this toxin.

The antihemolytic effect of the sumac tannins against hemolytic bacteria may be connected with precipitation of their toxins as well as with stabilization of erythrocyte membrane, preventing incorporation of toxins. The literature contains a sufficient number of findings about neutralization of bacterial toxins by polyphenols in models

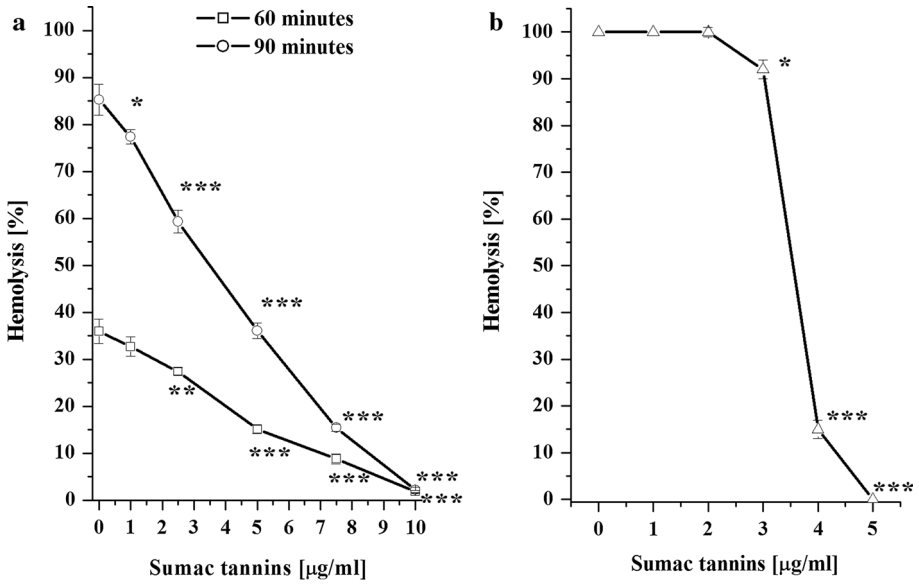

Fig. 2 Effects of the sumac tannin against hemolysis stimulated by incubation of human erythrocytes with *B. cereus* (**a**) and isolated hemolysin II (**b**). The data presented are the mean ± SE ($n = 9$). The effect of the sumac tannin was statistically significant according to one-way ANOVA test (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Experiment conditions are described in the "Materials and Methods" section

in vivo and in vitro (Choi et al. 2007; Hisano et al. 2003; Verhelst et al. 2013). It was established previously that high-molecular-weight polyphenols from Applephenon and hop bract tannins interacted with cholera toxin (AB_5), resulting in a large aggregate preventing toxin binding and its internalization in Vero cells isolated from kidney (Morinaga et al. 2005). The same authors demonstrated that resveratrol slightly precipitating cholera toxin did not suppress its binding to Vero cells but inhibited its internalization in the cells. These results suggest neutralization of cholera toxin by resveratrol at a membrane level (Morinaga et al. 2010).

The ability of high-molecular-weight tannins to form a large precipitating complex with α-toxin was also shown (Choi et al. 2007). However, the same authors also found that polyphenols inhibited rabbit erythrocyte hemolysis caused by *S. aureus* α-toxin, and an inhibiting effect was observed even if erythrocytes pretreated with tannins were washed before administration of α-toxin.

We also observed in our experiment that pretreatment of erythrocytes with sumac tannins followed by washing with PBS inhibited hemolysis induced by hemolysin II (data not presented). Retaining antihemolytic activity against hemolysin II even after washing erythrocytes indicated the strong incorporation of the sumac tannins in the erythrocyte membrane which can alter their physical parameters.

We studied the effect of the sumac tannins on erythrocyte structure using a Laurdan probe, allowing determination of the fluidity of the hydrophilic region of the lipid bilayer. This implies that the sumac tannins at the range of concentrations 0.75–5 μg/ml significantly increased GP of Laurdan probe incorporated in swine erythrocytes (Fig. 3). At the concentrations 0.75, 1.2, 2.2, 3.1, and 5 μg/ml, the

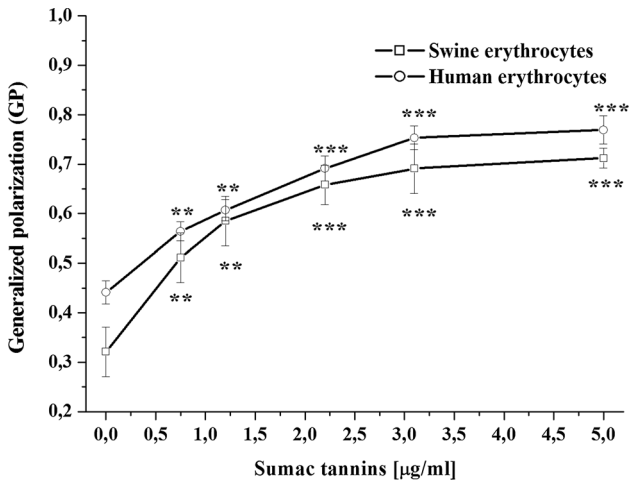

Fig. 3 Effects of the sumac tannin on erythrocytes membrane fluidity measured as generalized polarization (GP) of Laurdan. The data presented are the mean ± SE ($n = 9$). The effect of the sumac tannin was statistically significant according to one-way ANOVA test (**$p < 0.01$; ***$p < 0.001$). Experiment conditions are described in the "Materials and Methods" section

sumac tannins increased the value of GP to 0.51 ± 0.05, 0.59 ± 0.05, 0.66 ± 0.06, 0.69 ± 0.05, and 0.71 ± 0.02 in comparison with the control (0.32 ± 0.05). As shown in Fig. 3, a similar curve was obtained for Laurdan probe incorporated in human erythrocytes. In this case, the sumac tannin in a dose-dependent manner caused an increase of GP of Laurdan, which was connected with an increase of packing order in the hydrophilic region of the erythrocyte membrane.

It should be noted that the sumac tannins increased the rigidity of the hydrophilic region of erythrocyte membrane

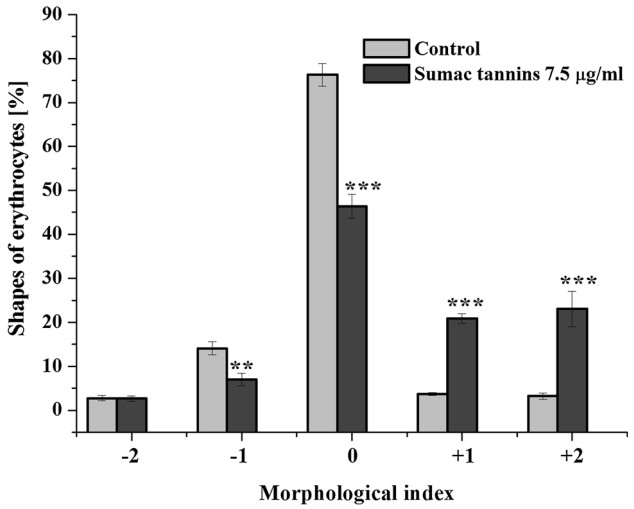

Fig. 4 Percent share of different shapes of swine erythrocytes in the presence (7.5 µg/ml; *dark gray bar*) and absence (*light gray bar*) of the sumac tannins. Morphological index: stomatocytes (−2), discostomatocytes (−1), discocytes (0), discoechinocytes (+1), and echinocytes (+2). Experiment conditions are described in the "Materials and Methods" section

in the concentration range in which they exhibit antihemolytic activity. The effect of the sumac tannins on the membrane structure of erythrocytes was shown by us previously using fluorescent probes of different membrane localizations. As was determined by TMA-DPH fluorescent probe, the sumac tannins significantly decreased surface membrane fluidity, but slightly elevated rigidity was noted using another fluorescent probe, DPH, which is localized in the membrane's hydrophobic region (Olchowik et al. 2012).

Embedding tannins in the outer layer of the erythrocyte membrane can cause changes in the shape and surface of cells. To confirm this interaction, we examined the impact of the sumac tannins on erythrocyte morphology using an optical microscope. Erythrocytes have a characteristic shape, called discocytes. Depending on the nature of the compounds and their location in the lipid bilayer, erythrocytes are transformed into echinocytes or stomatocytes. The compounds located in the outer layer form echinocytes, while compounds located in inner monolayer form stomatocytes. The percentages of various forms of erythrocytes in the control samples and samples treated with sumac tannins at the concentration of 7.5 µg/ml are shown in Fig. 4. It was found that the sumac tannins at the concentration 7.5 µg/ml significantly decreased the number of discostomatocytes and discocytes to 7.00 ± 1.44 and 46.40 ± 2.75 % in comparison with the control samples (14.07 ± 1.49 and 76.34 ± 2.59 %), at the same time increasing the level of discoechinocytes and echinocytes to 20.87 ± 1.09 and 23.07 ± 4.04 % compared with the control (3.67 ± 0.35 and 3.20 ± 0.69 %; Fig. 4).

Table 1 Effects of the sumac tannins on the growth of bacteria

Species of bacteria	MIC (mg/ml)
Bacillus cereus	1
Pseudomonas aeruginosa	4

It follows from the above that the antihemolytic effect of the sumac tannins against *B. cereus*- and *P. aeruginosa*-induced hemolysis seems to be connected to a greater extent with modification of the erythrocyte membrane structure. The difference in antihemolytic activity of the sumac tannins toward the two bacterial species is likely to be related to different mechanisms of action of *B. cereus* and *P. aeruginosa* toxins on erythrocyte membranes. *B. cereus* produces toxins forming pores with various internal dimensions of the channels from monomers directly in the membrane of erythrocytes (Baida and Kuzmin 1996; Lund et al. 2000; Miles et al. 2002; Andreeva et al. 2007; Andreeva-Kovalevskaya et al. 2008). The hemolytic activity of *P. aeruginosa* is mainly mediated by toxins which are directly transported to the target cell via the type 3 secreting system (T3SS; Galle et al. 2012). The sumac tannins apparently do not prevent the incorporation of the *B. cereus* toxin monomers into the erythrocyte membrane, but owing to inducing membrane rigidity, they prevent their lateral diffusion and, consequently, formation of a pore. However, the incorporation of the sumac tannins into erythrocyte membranes which contribute to increasing their outlayer surface poorly limits *P. aeruginosa* secreting complex III interaction with erythrocytes and accordingly inhibits hemolysis to a lesser extent. But at the same time, we cannot exclude that the antihemolytic activity of the sumac tannins is related to some extent to their direct interaction with bacteria.

Antibacterial Activity

We studied also interaction of the sumac tannins with bacteria in an antimicrobial test. Our data presented in Table 1 indicate that the antimicrobial effect of the sumac tannins depended on the bacterial type.

Thus, MIC for *P. aeruginosa* was four times higher than for *B. cereus*, which suggests a weaker effect of the sumac tannins on Gram-negative bacteria. In order to determine what caused the specificity of antimicrobial action of sumac tannins, we studied their effects on structure bacterial membranes.

The fluidity of membrane was determined by means of fluorescence anisotropy changes of fluorescent probes with different localizations in membranes—DPH in hydrophobic

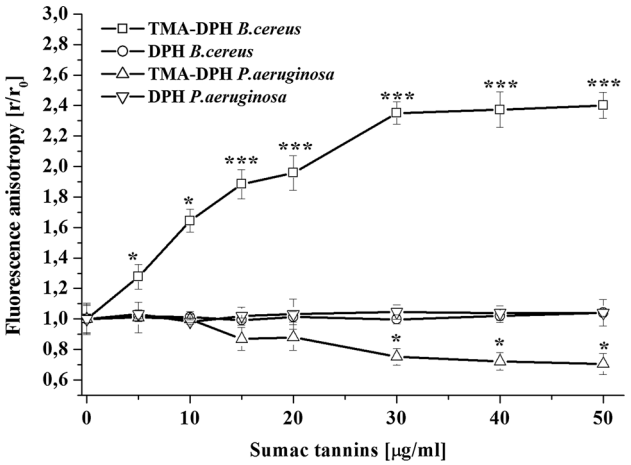

Fig. 5 Effect of the sumac tannins on fluorescence anisotropy of TMA-DPH and DPH in membranes of *B. cereus* and *P. aeruginosa*. The data presented are the mean ± SE ($n = 9$). The effect of the sumac tannin was statistically significant according to one-way ANOVA test (*$p < 0.05$; ***$p < 0.001$). Experiment conditions are described in the "Materials and Methods" section

core and TMA-DPH at the aqueous membrane interface (Mykytczuk et al. 2007). The results are present as the ratio (r/r_o) between the fluorescence anisotropy of probes in the presence (r) and absence (r_o) of the sumac tannins. We found that, consistent with their hydrophilic properties, sumac tannins did not cause statistically significant changes in fluorescence anisotropy of DPH (i.e., the packing hydrophobic acyl chains of membranes phospholipids) of both bacterial species (Fig. 5).

Our measurements of TMA-DPH probe fluorescence anisotropy demonstrated that sumac tannins are capable of affecting the fluidity of aqueous membrane interface of bacteria. However, the magnitude and the direction of changes elicited in their presence in Gram-positive *B. cereus* and Gram-negative *P. aeruginosa* were all quite different. Namely, in the tested concentrations, sumac tannins (5–50 μg/ml) strongly increased the r/r ratio (indicates an intensive decrease membrane fluidity) of the probe in the membranes of *B. cereus*. At 50 μg/ml, the anisotropy of TMA-DPH fluorescence increased by 140.06 ± 8.58 %. In the case of *P. aeruginosa*, tannins exerted an opposite effect and decreased of TMA-DPH fluorescence anisotropy, suggesting an increase in membrane fluidity. However, statistically significant effects were observed only at substantially higher concentrations of tannins (30–50 μg/ml). Even at 50 μg/ml of sumac tannin, TMA-DPH fluorescence anisotropy decreased only by 29.47 ± 6.94 % as compared to the control. This relative efficacy of tannins in inducing changes in membrane fluidity correlates with their above-noted antimicrobial activity (see Table 1). The observed increase in rigidity of *B. cereus* membranes in the presence of tannins may result in diminished release of pore-forming toxin. For example, decreased secretion of α-toxin and coagulase from *S. aureus* in the presence of epicatechin gallate, supposedly due to increase in rigidity of bacterial membrane, was shown previously by Shah et al. (2008). This mechanism could, at least in part, account for the protective effects of tannins against hemolysis induced in erythrocytes by *B. cereus* reported here. However, in our study, sumac tannins effectively inhibited hemolysis induced both by bacteria and by purified toxin. The latter finding suggests that the mechanisms responsible for antihemolytic effects of sumac tannins are due to the modification of the erythrocyte membranes structure rather than those on bacteria.

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Conflict of interest All authors declare no conflict of interest.

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