The Mechanisms of Lipid Vesicle Fusion Inhibition by Extracts of Chaga and Buckthorn Leaves

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Abstract—The ability of extracts of grapefruit seeds (ESG), sea buckthorn leaves (ESBL), and chaga (EC) to inhibit membrane fusion was evaluated. It was found that ESBL and EC inhibited Ca^{2+}-mediated fusion of phosphatidylglycerol-enriched lipid vesicles; the inhibition indexes were about 90 and 100%, respectively. ESG did not inhibit the fusion of negatively charged liposomes induced by calcium. In addition to calcium-mediated liposome fusion, EC inhibited the fusion of vesicles from a mixture of phosphatidylcholine and cholesterol under the action of polyethylene glycol with a molecular weight of 8000 Da (the inhibition index was 80%). The other two extracts had no effect on polymer-induced fusion of uncharged membranes. The effect of some major components of the tested extracts on the fusion of vesicles was evaluated. It has been shown that flavonols, quercetin and myricetin, which are major components of ESBL, inhibited the fusion of negatively charged membranes under the action of calcium (the inhibition indexes were about 85 and 60%, respectively). Another flavonol of ESBL, the glycoside of quercetin rutin, did not have such an effect. The data obtained made it possible to relate the ESBL suppression of calcium-induced fusion of lipid vesicles with the presence of quercetin and myricetin in its composition. These flavonols had virtually no effect on polyethylene glycol-induced vesicle fusion, which is consistent with the absence of ESBL action on liposome fusion under the action of polymer. The ability of quercetin and myricetin to reduce the melting temperature of phosphatidylglycerol with saturated hydrocarbon chains and to increase the half-width of the peak corresponding to melting has been demonstrated. The observed correlation between the parameters characterizing the thermotropic behavior of the lipid in the presence of quercetin and myricetin and the index of inhibition of calcium-mediated liposome fusion by these compounds may indicate a relationship between the ability of flavonols to influence the packaging of membrane lipids and inhibit vesicle fusion. Pentacyclic triterpenoids, betulin and lupeol, which are part of EC, did not inhibit the fusion of vesicles under the action of both calcium and polyethylene glycol, and their presence in EC cannot be responsible for the antifusogenic activity of EC.

Keywords: natural extracts, membrane fusion inhibitors, liposomes, flavonols, saponins

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INTRODUCTION

Natural extracts are widely used in medicine as independent medications and in combination with other biologically active compounds. In addition, the extracts are actively used as components of cosmetics, since they have pronounced anti-inflammatory and antimicrobial effects.

Published data indicate a high antiviral activity of grapefruit seed extract (EGS). It has been established that EGS has a significant potential for use in poultry farming as a disinfectant, since it significantly reduced the titer of viruses that cause infectious diseases in poultry, in particular, avian influenza and Newcastle disease [1]. The results of recently published studies have shown that a commercially available nasal spray containing EGS can be used as an additional therapy for COVID-19 of mild and moderate severity [2].

It is known that sea buckthorn exhibits antiviral properties against the Dengue virus [3, 4]. Sea buckthorn leaf extract (ESBL) proved to be as effective in maintaining the viability of cells infected with dengue virus as the well-known antiviral drug ribavirin [5]. ESBL has also been shown to exhibit activity comparable to oseltamivir against influenza A and B viruses [6].

Recently, the antiviral activity, including against SARS-CoV-2, of birch fungus, chaga, has been widely discussed [7, 8]. The results of molecular docking

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Abbreviations: DOPC, 1,2-dioleyl-*sn*-glycero-3-phosphocholine; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol); CHOL, cholesterol; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol); PEG-8000, polyethylene glycol with a molecular weight of 8000 Da; ESG, extracts of grapefruit seeds; ESBL, extracts of sea buckthorn leaves; EC, extracts of chaga.

showed that the components of the chaga extract (EC) (beta-glucan, galactomannan, and betulinic acid) bind to the C-terminal fragment of the receptor-binding domain of the SARS-CoV-2 S-protein [9]. EC also inhibits the fusion of the type 1 Herpes simplex virus with the cell membrane [10]. It was found that EC exhibits antiviral activity against a large number of viruses that cause diseases in cats: calicivirus, Herpes type 1, H3N2 and H5N6 influenza, panleukopenia, infectious peritonitis and immunodeficiency [11, 12]. A study of the mechanism of antiviral action of EC against feline calicivirus has shown that the inhibitory effect of the extract is associated with blocking the binding/absorption of the virus [11]. The activity of EC against the human immunodeficiency virus has also been demonstrated [13].

It is obvious that the antiviral activity of natural extracts should be due to their specific chemical composition. It is known that the composition of EGS in large quantities includes flavanone glycosides, naringin, hesperidin, and narirutin [14]. Naringin and narirutin are glycosides of naringenin, and hesperidin is a glycoside of a close analogue of naringenin, hesperetin. Analysis of the literature data showed that ESBL are rich in flavonols, quercetin, myricetin, and kaempferol, as well as various quercetin glycosides, in particular, rutin (quercetin-3-rutinoside) [15−17]. To date, about forty lanostane triterpenoids have been identified in the composition of EC, primarily lanosterol and its derivative, inotodiol. Pentacyclic triterpenoids, such as betulin and lupeol, as well as other sterols, in particular ergosterol, were found in several times lower concentrations than tetracyclic triterpenoids in the composition of chaga [18].

In this study, the ability of EGS, ESBL, and EC to inhibit the fusion of negatively charged and uncharged lipid vesicles under the action of calcium and high-molecular polyethylene glycol, respectively, was evaluated. The role of some components of extracts in the liposome fusion inhibition was determined; it was also established which physical and chemical properties of the bilayer are responsible for inhibiting membrane fusion by the components of extracts.

MATERIALS AND METHODS

The following reagents were used in the work: sodium chloride (NaCl), HEPES, NaOH, ethanol, dimethyl sulfoxide (DMSO), Triton X-100, Sephadex G-50, calcein, sorbitol, calcium chloride (CaCl$_2$), polyethylene glycol with a molecular weight of 8000 Da (PEG-8000), quercetin, myricetin, rutin, betulin, lupeol, 1,2-dioleyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (DOPG), cholesterol (CHOL), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (DPPG) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanol-amine-N-(lissamine rhodamine) from Sigma (USA).

Grapefruit seed extract (EGS), sea buckthorn leaf extract (ESBL) and chaga extract (EC) were provided by Evalar CJSC. Testing was carried out for three samples of each extract, representing independent extraction series.

Investigation of the ability of the tested extracts and their components to inhibit the fusion of lipid vesicles mediated by various inducers. Monolamellar lipid vesicles from mixtures of DOPC/CHOL (80/20 mol %) and DOPC/DOPG/CHOL (40/40/20 mol %) loaded with a fluorescent marker calcein were formed using a mini extruder from Avanti Polar Lipids (USA). The solutions of lipids in chloroform were placed in a vial and the solvent was removed by a nitrogen stream. The resulting lipid film was hydrated with a buffer (35 mM calcein, 10 mM HEPES-NaOH, pH 7.4) and after five times freezing−thawing the mixture was passed through a polycarbonate membrane (Nucleopore TM, USA) with a pore diameter of 100 nm for 13 times to obtain a homogeneous population of large single-layer liposomes. The calcein that was not entrapped in the vesicles was removed by gel filtration on a column with Sephadex G-50. A calcein-free buffer (150 mM NaCl, 10 mM HEPES-NaOH, pH 7.4) was used as an eluent. Fluorescence of calcein inside liposomes at a concentration of 35 mM was self-quenched. Calcium (40 mM CaCl$_2$) and polyethylene glycol with a molecular weight of 8000 Da (PEG-8000 at a concentration of 20 wt %) were used to induce the fusion of DOPC/DOPG/CHOL and DOPC/CHOL liposomes, respectively [19−22].

The samples of extracts from the initial solutions in water or DMSO were added into a suspension of liposomes up to a concentration of 100 μg/mL.

The fluorescence intensity of calcein outflowing from the liposomes into the solution medium during their fusion was measured with a Fluorat Panorama-02 spectrofluorimeter (at an excitation wavelength of 490 nm and emission of 520 nm). At the end of each experiment, Triton X-100 was introduced into the solution. At a concentration of 10 mM, this detergent destroyed all liposomes in the suspension, releasing the marker trapped by vesicles into the medium.

The index of inhibition of lipid vesicle fusion (*II*) by the tested extracts was calculated as:

$$II = \frac{RF_{\text{Ind}} - RF_{\text{Inh}}}{RF_{\text{Ind}}} \times 100\%, \qquad (1)$$

where RF_{Ind} and RF_{Inh} represented the average maximum leakage of the fluorescent marker from vesicles induced by the introduction of calcium chloride or PEG-8000 in the absence and in the presence of the tested extracts, respectively.

The values of RF (%) (RF_{Ind} and RF_{Inh}) were determined by the formula:

$$RF = \frac{I_{\text{i}} - I_0}{1.1 I_{\text{max}} - I_0} \times 100\%, \qquad (2)$$

where I_i and I_0 were the fluorescence intensities of the solution in the presence and absence of the tested extracts, I_{max} was the fluorescence intensity of the solution after the addition of Triton X-100 (multiplier 1.1 was introduced to account for dilution of the sample with an aqueous detergent solution). RF_{Inh} was evaluated considering a possible intrinsic effect of extracts on the permeability of liposomes for the marker (calcein leakage due to the disordering of lipids under the action of extracts).

The kinetics of marker release under the action of fusion inducers before and after the introduction of the tested extracts was characterized by the time of e-fold increase in relative fluorescence.

For each type of extract, nine independent repetitions were performed with samples from three different batches. The parameters characterizing the effect of individual components of extracts on membrane fusion were determined by calculating the arithmetic mean values obtained in 2−3 independent experiments.

To prove the statistical significance of the detected differences in the average RF values before and after the addition of extracts or their components, the non-parametric Mann−Whitney−Wilcoxon criterion ($*p \leq 0.01$) was used.

Confocal fluorescence microscopy of giant liposomes. Visualization of changes in the behavior of vesicles before and after the introduction of PEG-8000 and chaga extract into the suspension was performed using confocal fluorescence microscopy. Giant unilamellar vesicles were prepared from the mixture of DOPC/CHOL (80/20 mol %) and 1 mol % fluorescent lipid probe 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(rhodamine lizzamine) by electroformation (standard protocol, 3 V, 10 Hz, 1 h, 25°C) using a commercially available Nanion vesicle prep pro device (Germany). The resulting suspension of liposomes contained 0.5 mM of lipid in 1 M sorbitol solution. To induce vesicle fusion, PEG-8000 was added into the suspension to a concentration of 10 wt % and incubated for 5−10 min at room temperature (25 ± 1°C). EC (35 µg/mL) and PEG-8000 (10 wt %) were sequentially added into the experimental samples with incubation at each stage for 10 min. Liposomes were observed through a 100×/1.4 HCX PL immersion lens in a Leica TCS SP5 Apo confocal laser system (Leica Microsystems, Germany). The observations were carried out at 25°C. Fluorescence of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(Rhodamine lizzamine) was excited at 543 nm (Helium−neon laser).

Differential scanning microcalorimetry of lipid vesicles modified with extract components. Giant unilamellar liposomes were formed from DPPG by electroformation using the Nanion vesicle prep pro device (Germany). An alternating voltage with an amplitude of 3 V and a frequency of 10 Hz was applied to the glasses

for 1 h at 55°C. The lipid concentration was 3 mM. Quercetin, myricetin, and rutin were added into the experimental samples until the lipid : flavonol ratio reached 10 : 1. Thermograms of liposomal suspensions were obtained using a µDSC7 differential scanning microcalorimeter (Setaram, France). Reproducibility of the temperature dependence of the heat capacity was achieved by reheating the sample immediately after cooling at a constant rate of 0.2°C/min. The peaks on the thermograms were characterized by the maximum temperature of the main phase transition of DPPG (T_m) and the half-width of the main peak ($T_{1/2}$). The changes in these parameters made it possible to evaluate the effect of flavonols on the packing density of membrane lipids.

The parameters characterizing the effect of flavonols on the thermotropic behavior of lipids were determined by calculating the arithmetic mean of the values obtained for 2 independent series of liposome preparation.

The values of RF, t, II, ΔT_m, and $\Delta T_{1/2}$ were presented as mean ± standard error of the mean (mean ± SE).

RESULTS AND DISCUSSION

Figure 1a shows the time dependence of the relative fluorescence intensity of calcein (RF, %) released from DOPC/DOPG/CHOL (40/40/20 mol %) liposomes during vesicle fusion induced by the introduction of 40 mM CaCl$_2$ into the suspension before and after incubation with EGS, ESBL, or EC at a concentration of 100 µg/mL for 30 min at room temperature. Table 1 shows the average values of the maximum calcein leakage (RF) during the fusion of DOPC/DOPG/CHOL vesicles under the action of calcium in the absence and in the presence of various extracts. The average maximum leakage of fluorophore during fusion of lipid vesicles unmodified with extracts upon addition of calcium chloride was about 90%. A decrease or increase in this value indicated the inhibition or activation of calcium-mediated fusion of negatively charged vesicles in the presence of the corresponding extract. As shown in Fig. 1a and Table 1, ESBL and EC significantly inhibited the fusion of DOPC/DOPG/CHOL vesicles caused by the addition of calcium chloride into the suspension: the corresponding RF values were 8 and 2%, respectively. To compare the antifusogenic activity of the extracts, their inhibition indices (II) were calculated based on the RF values according to formula (1) (Table 1). In the case of ESBL, II was about 90%, and in the case of EC it reached almost 100% (Table 1). EGS did not inhibit calcium-mediated fusion of negatively charged liposomes (Fig. 1a and Table 1).

Figure 1b shows the time dependence of the relative fluorescence intensity of calcein (RF, %) resulting from the fusion of DOPC/CHOL (80/20 mol %) vesicles after addition of 20 wt % PEG-8000 before and
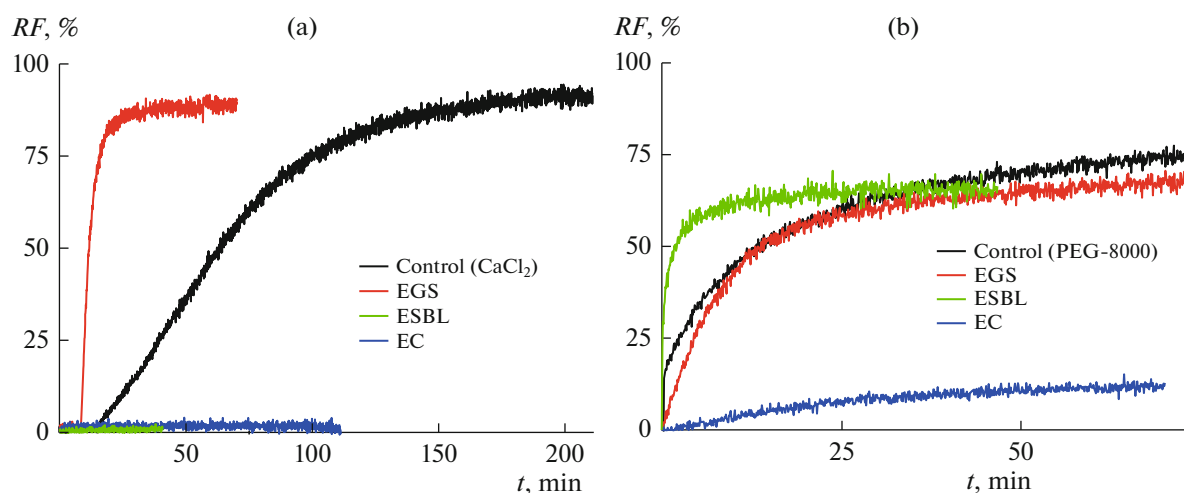
Fig. 1. Time course of the relative fluorescence intensity of calcein (*RF*, %) leaking upon (a) calcium-mediated fusion of DOPC/DOPG/CHOL (40/40/20 mol %) liposomes and (b) PEG-8000-induced fusion of DOPC/CHOL (80/20 mol %) vesicles in the control conditions (*black line*) and after incubation with 100 μg/mL of EGS (*red line*), ESBL (*green line*), and EC (*blue line*).

after incubation with 100 μg/mL of EGS, ESBL, or EC. The average maximum leakage during fusion of unmodified liposomes extracts upon the introduction of PEG-8000 in the absence of extracts was 75% (Table 1). As is shown in Fig. 1b and Table 1, EGS and ESBL did not suppress PEG-induced fusion of uncharged vesicles: the corresponding *RF* values (79 and 72%, respectively) were close to the control value. At the same time, as in the case of calcium-induced fusion of negatively charged liposomes, EC suppressed the fusion of uncharged vesicles under the

action of PEG-8000: *RF* was about 10%, and *II* exceeded 70% (Table 1).

Table 1 also presents the characteristic release times of the marker during liposome fusion in the absence and in the presence of the extracts. It can be seen that the time constant characterizing the kinetics of marker release during the fusion of DOPC/DOPG/CHOL (40/40/20 mol %) and DOPC/CHOL (80/20 mol %) vesicles under the action of calcium and PEG, was about 60 and 50 min, respectively. Notably, the fusion of DOPC/DOPG/CHOL

Table 1. Parameters characterizing the effect of the extracts at a concentration of 100 μg/mL on calcein leakage at the fusion of vesicles formed from mixtures of DOPC/DOPG/CHOL (40/40/20 mol %) and DOPC/CHOL (80/20 mol %) upon the action of 40 mM CaCl$_2$ and 20 wt % PEG-8000, respectively

Extract	*RF*, %	*t*, min	*II*, %	*RF*, %	*t*, min	*II*, %
	CaCl$_2$			PEG-8000		
−	90 ± 6	61 ± 6	−	75 ± 5	46 ± 8	−
EGS	92 ± 5	9 ± 5	−3 ± 2	73 ± 6	33 ± 5	−10 ± 5
ESBL	8 ± 3*	−#	90 ± 3	72 ± 7	4 ± 2	−8 ± 9
EC	2 ± 1*	−#	97 ± 3	13 ± 5*	−#	79 ± 3

RF is the maximum leakage of the fluorescent marker at the fusion of liposomes formed from mixtures of DOPC/DOPG/CHOL (40/40/20 mol %) and DOPC/CHOL (80/20 mol %), upon addition to the suspension of 40 mM CaCl$_2$ and 20 wt % PEG-8000 in the absence (−) and in the presence of the tested extracts at a concentration of 100 μg/mL; *t* is the time constant characterizing the kinetics of marker release during vesicle fusion (the dependence of marker leakage on time is approximated by a one-exponential function); *II* is the index of inhibition by extracts of calcium and PEG-8000-mediated fusion of lipid vesicles of different composition.
#Evaluation was not carried out due to the smallness of the *RF* value. *, $p \leq 0.01$ (Mann−Whitney−Wilcoxon criterion, comparison of calcium- and PEG-8000-induced dye leakage from the vesicles in the absence and in the presence of extracts).
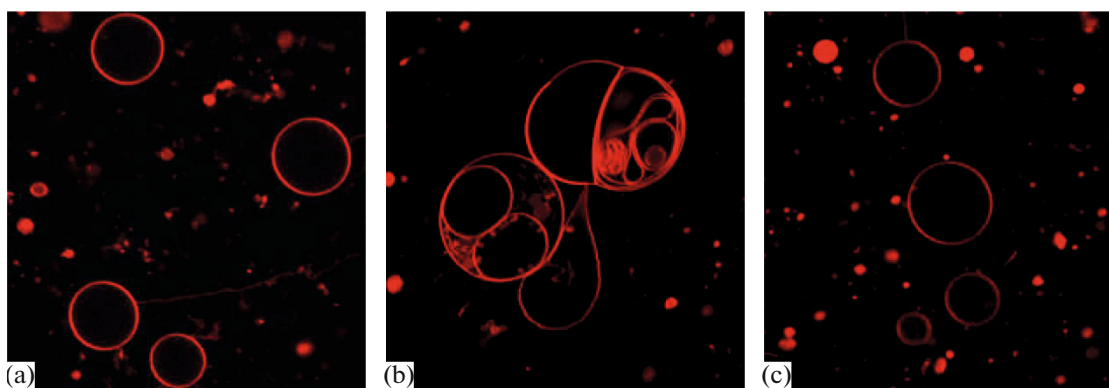
Fig. 2. Examples of fluorescent microphotographs of giant vesicles formed from the mixture of DOPC/CHOL (80/20 mol %) and 1 mol % of fluorescent lipid probe, dipalmitoyl phosphatidylethanolamine-N-(lissamine rhodamine B sulfonyl), in the absence of the agents (a), in the presence of 10 wt % PEG-8000 (b), and after addition of 10 wt % PEG-8000 into the suspension of liposomes pretreated with 35 μg/mL of EC (c). The size of each micrograph is 65 × 65 μm.

(40/40/20 mol %) liposomes under the action of calcium in the presence of EGS and the fusion of DOPC/CHOL (80/20 mol %) vesicles under PEG-8000 in the presence of ESBL was accelerated several times. These differences can be explained by the acceleration of adsorption of the corresponding fusion inductors on the membranes modified by the tested extracts.

Confocal fluorescence microscopy of giant unilamellar vesicles formed from the mixture of DOPC/CHOL (80/20 mol %) was performed to visualize the fusion of vesicles under the action of PEG-8000 and its inhibition by EC. Figure 2 presents fluorescent micrographs of DOPC/CHOL liposomes in the absence of any agent (Fig. 2a), in the presence of 10 wt % PEG-8000 (Fig. 2b), and after the application of 10 wt % PEG-8000 to liposomes, pre-modified with 35 μg/mL of EC (Fig. 2c). PEG-8000 caused an increase in the size of liposomes, their deformation and induced the formation of multilayer and multivesicular liposomes (Fig. 2b); this indicated a high efficiency of fusion of lipid vesicles under the action of the polymer. At the same time, as Fig. 2c shows, PEG-8000 did not have a similar effect on the lipid vesicles treated with EC: the probability of the appearance of multilamellar structures was low, and single monolamellar vesicles were found in large quantities in the suspension. These data supported the conclusion that EC suppresses PEG-8000-induced fusion of liposomes.

To elucidate the mechanisms of inhibition of calcium- and PEG-induced vesicle fusion by ESBL and EC, we evaluated the effect on this process of some major components of extracts: flavonols contained in ESBL; quercetin, rutin and myricetin, as well as pentacyclic triterpenoids found in the composition of EC—betulin and lupeol. The chemical structures of the tested compounds are shown in Fig. 3.

Figure 4 and Table 2 summarize the data obtained in the study of the antifusogenic activity of the tested components of ESBL and EC. Figure 4a and Table 2 show that the ESBL flavonols, quercetin and myricetin, inhibited the fusion of negatively charged membranes under the action of calcium (*II* was about 85 and 60%, respectively). Other component of ESBL—quercetin glycoside rutin—did not cause such an effect. Thus, the suppression of calcium-induced fusion of lipid vesicles under the action of ESBL can be attributed to the presence of quercetin and myricetin in its composition. The inability of flavonols to inhibit the fusion of uncharged liposomes under the action of PEG-8000 (Fig. 4b and Table 2) was in good agreement with the absence of ESBL effect on this process (Table 1).

To understand the relationship between the effect of ESBL components on the fusion of negatively charged liposomes enriched with phosphatidylglycerol and their ability to modify the physical properties of phosphatidylglycerol-containing membranes, the thermotropic behavior of DPPG was studied before and after the introduction of quercetin, myricetin, or rutin into the liposomal suspension. In the absence of flavonols, the temperature of the main phase transition of DPPG, T_m, was 41.3°C, the half-width of the main peak, $T_{1/2}$, was 0.6°C (Fig. 5). The main parameters characterizing the melting of DPPG in the presence of flavonols are presented in Table 2. Quercetin and myricetin at the lipid : flavonol ratio of 10 : 1 lowered T_m to by 1.2 and 1.1°C, respectively. At the same time, quercetin increased $T_{1/2}$ by 0.8°C, and myricetin increased $T_{1/2}$ by 0.6°C (Table 2). The introduction of rutin at the same concentration did not change the thermotropic behavior of DPPG (Table 2). The observed correlation between the parameters characterizing the melting of DPPG in the presence of flavonols and their antifusogenic activity in calcium-induced fusion of phosphatidylglycerol-containing
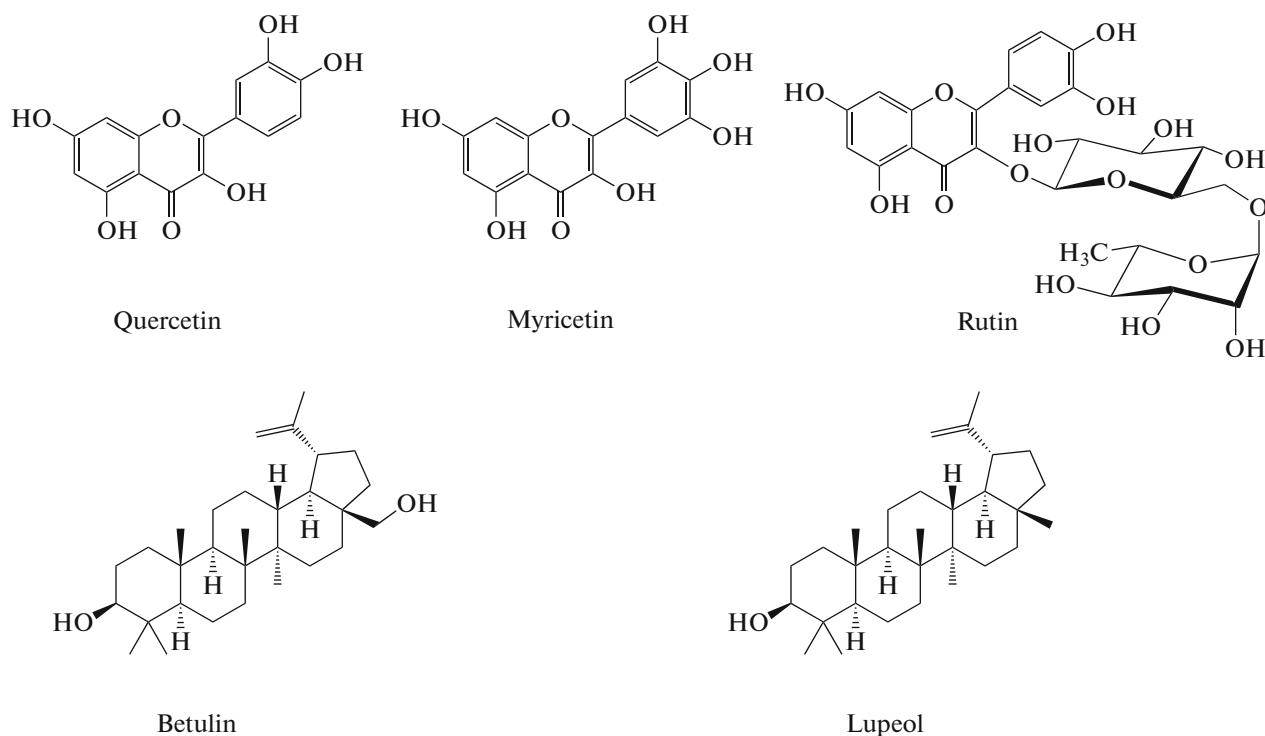
Fig. 3. The chemical structure of some components of ESBL and EC: quercetin, myricetin, rutin, betulin, and lupeol.
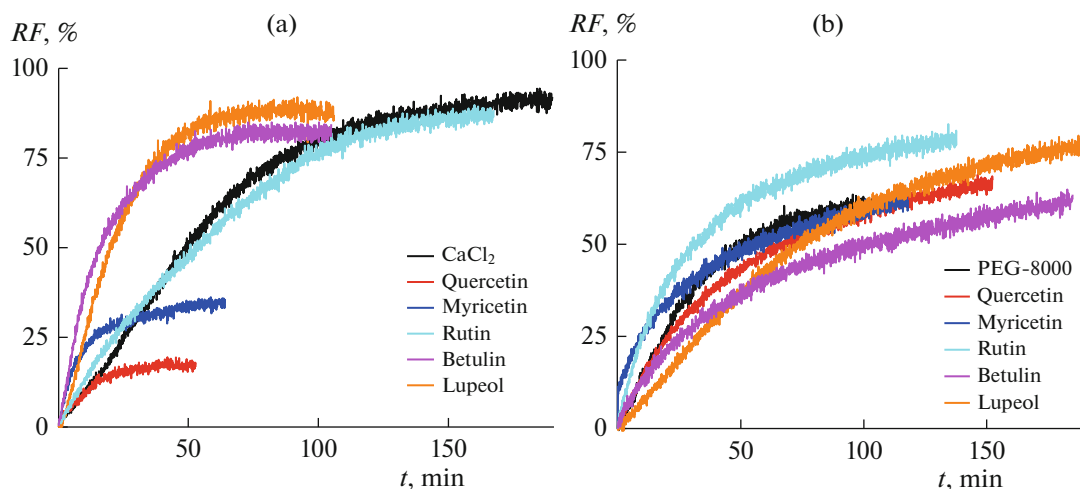

Fig. 4. Time course of the relative fluorescence intensity (RF, %) of calcein leaking upon (a) calcium-mediated fusion of DOPC/DOPG/CHOL (40/40/20 mol %) liposomes and (b) PEG-8000-induced fusion of DOPC/CHOL (80/20 mol %) vesicles in the control conditions (*black line*) and after incubation with 20 μM of flavonols, quercetin (*red line*), myricetin (*blue line*), or rutin (*cyan line*) and 10 μM of pentacyclic triterpenoids, betulin (*purple line*), or lupeol (*orange line*).

vesicles (Table 2) indicated the relationship between their ability to affect the packing density of membrane-forming lipids and inhibit liposome fusion. A similar conclusion was made when the effect of alkaloids on the calcium-mediated fusion of vesicles and the thermotropic behavior of lipids were compared [23].

We have previously shown that quercetin and myricetin have a less pronounced effect on the ther-motropic behavior of dipalmitoylphosphocholine (DPPC) than DPPG: at the DPPC : flavonol ratio of 10 : 1 quercetin and myricetin caused a drop of T_m by 0.3°C, and an increase in $T_{1/2}$ by 0.7 and 0.2°C, respectively [24]. As in the case of DPPG, rutin had no effect on the melting of DPPC [24]. The obtained data on the significant suppression of calcium-medi-

Table 2. Parameters characterizing the effect of the components of the tested extracts on calcein leakage in the course of fusion of the vesicles formed from mixtures of DOPC/DOPG/CHOL (40/40/20 mol %) and DOPC/CHOL (80/20 mol %), upon the action of 40 mM $CaCl_2$ and 20 wt % PEG-8000, respectively

Component, µM	RF, %	II, %	RF, %	II, %	$-\Delta T_m$, °C	$\Delta T_{1/2}$, °C
	$CaCl_2$		PEG-8000		DPPG	
–	90 ± 6	–	75 ± 5	–	–	–
Quercetin, 20	9 ± 1*	85 ± 2	77 ± 11	−2 ± 5	1.2 ± 0.2	0.8 ± 0.1
Myricetin, 20	31 ± 3*	58 ± 4	76 ± 12	3 ± 10	1.1 ± 0.2	0.6 ± 0.2
Rutin, 20	88 ± 1	−5 ± 2	80 ± 3	−7 ± 6	0	0
Betulin, 10	90 ± 2	1 ± 1	67 ± 5	11 ± 2	0.1 ± 0.1[a]	0.1 ± 0.1[a]
Lupeol, 10	82 ± 9	7 ± 8	70 ± 4	8 ± 2	0.1 ± 0.1[a]	0.2 ± 0.1[a]

[a] According to [25]; RF is the maximum leakage of the fluorescent marker at the liposome fusion; II is the index of inhibition by major components of the tested extracts of calcium- and PEG-8000-mediated vesicle fusion; ΔT_m and $\Delta T_{1/2}$ are changes in temperature and half-width of the peak of the main phase transition of DPPG ((lipid : flavonol and lipid : triterpenoid ratios were 10 : 1 and 50 : 1, respectively); *, $p \leq 0.01$ (Mann–Whitney–Wilcoxon criterion, comparison of calcium- or PEG-8000-induced dye leakage from the vesicles in the absence and in the presence of extract components).

ated fusion of phosphatidylglycerol-containing vesicles in the presence of quercetin and myricetin and the absence of the inhibitory ability of these flavonols against PEG-8000-induced fusion of phosphatidylcholine-enriched vesicles (Table 2), the pronounced effect of quercetin and myricetin on DPPG melting (Table 2), and the reduced effect in the case of DPPC [24] suggest that the antifusogenic activity of these flavonols may be due to their ability to disorder phosphatidylglycerol membranes. At the same time, the less pronounced effect of quercetin and myricetin on the packing density of phosphatidylcholine bilayers led to the inability of flavonols to suppress the fusion of phosphatidylcholine-enriched vesicles.

Figure 4 shows that pentacyclic triterpenoids, which are part of EC, betulin and lupeol, practically did not inhibit the fusion of either negatively charged vesicles under the action of calcium (Fig. 4a) or uncharged liposomes in the presence of PEG-8000 (Fig. 4b). Thus, due to the unexpressed antifusogenic activity of these triterpenoids in both systems (Table 2), betulin and lupeol could not be the components responsible for the EC suppression of vesicle fusion under the action of both calcium and PEG-8000 (Table 1). It should be noted that the tested triterpenoids did not affect significantly the thermotropic behavior of DPPG (T_m decreased by less than 0.3°C, and $T_{1/2}$ increased by 0.1°C (Table 2)) and DPPC [25]. This is in a good agreement with the assumption that the disordering ability of the tested compounds is related to their antifusogenic activity: the weak effect of

betulin and lupeol on the melting of DPPG (Table 2) and DPPC [25] manifested in the inability of these triterpenoids to inhibit the fusion of phosphatidylglycerol- and phosphatidylcholine-enriched vesicles (Table 2).

It is unlikely that the antifusogenic ability of EC was due to the presence of sterols in its composition since they are known for their ability to increase the negative spontaneous curvature of lipid monolayers during adsorption [26] and thereby can rather induce membrane fusion. The presence of a hydroxyl group in
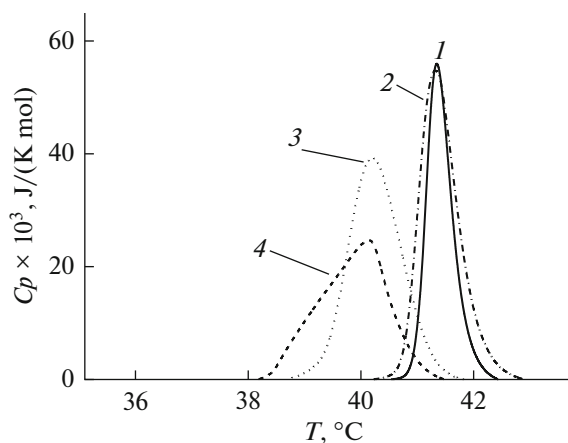

Fig. 5. The thermograms of DPPG ($Cp(T)$) in the absence (1) and in the presence of rutin (2), myricetin (3), or quercetin (4). Lipid : flavonol ratio was 10 : 1.

the side chain of a hydrophilic derivative of lanosterol, inotodiol, could prevent its immersion in the hydrocarbon backbone of the bilayer and change the transmembrane profile of lateral pressure in such a way as to increase the energy of formation of intermediates of fusion and suppress the process of membrane unification. Inhibition of fusion of uncharged lipid vesicles by chaga extract may indicate the antiviral effect of this extract.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

This article does not contain any studies involving animals or human participants performed by any of the authors.

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