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NATURAL PRESERVING ADDITIVES FOR COSMETIC COMPOSITIONS

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Abstract. At present time functional food, medicine and cosmetic products, bioactive additives in the form of natural extracts usage is being increased. As a raw material for their obtaining the justified decision is renewable biomass application in particular, of hanging birch (*Bétula péndula*) and black currant (*Ríbes nígrum*) vegetative organs naturally growing in the Krasnoyarsk region. The research authors found out that black currant leaves water-alcohol extract is the most effective preservative. Birch leaves water-alcohol extract has somewhat lower biocide activity. It was detected that sodium benzoate blocks fungal culture Candida albicans growth more effective in neutral than in acidic environment and it allows to recommend black currant and birch leaves extract application in combination with sodium benzoate additives to strengthen conserving system fungicidal activity. Low toxicity in relation to sodium benzoate and currant leaves extract cell fiber cultures and birch leaves and propolis some toxicity was confirmed. The obtained results let recommend currant leaves extract as the least toxic biocide natural additive while birch leaves extract can be used only in certain (minimal) concentrations.

Keywords: BAS, natural extract, preservative, biocidal additives, leaves of *Ribes nigrum L.* sort*,* cosmetic product ingredient.

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НАТУРАЛЬНЫЕ КОНСЕРВИРУЮЩИЕ ДОБАВКИ ДЛЯ КОСМЕТИЧЕСКИХ СОСТАВОВ

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Аннотация. В настоящее время расширяется использование продуктов функционального питания, лекарственных и косметических средств, биоактивных добавок в виде натуральных экстрактов. В качестве сырья для их получения оправданным решением является использование возобновляемой биомассы, в частности вегетативных органов березы повислой (*Bétula péndula*) и черной смородины (*Ríbes nígrum*), произрастающих в естественных условиях в Красноярском крае. Авторами исследования установлено, что наиболее эффективным консервантом является водно-спиртовой экстракт листьев черной смородины. Водно-спиртовой экстракт листьев березы повислой обладает несколько меньшей биоцидной активностью. Выявлено, что бензоат натрия более эффективно блокирует рост грибковой культуры *Candida albicans* в нейтральной, чем в кислой среде, что позволяет

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рекомендовать применение экстракта листьев черной смородины и березы в сочетании с добавками бензоата натрия для усиления фунгицидной активности консервирующей системы. Доказана низкая токсичность бензоата натрия и экстракта листьев черной смородины по отношению к культурам клеточных волокон. Одновременно выявлена некоторая токсичность по отношению к клеточной культуре экстрактов листьев березы повислой. Полученные результаты позволяют рекомендовать экстракт листьев черной смородины как наименее токсичную природную биоцидную добавку, в то время как экстракт листьев березы повислой можно использовать только в определенных (минимальных) концентрациях.

Ключевые слова: БАВ, натуральный экстракт, консервант, биоцидные добавки, листья черной смородины *Ribes nigrum L*., ингредиент косметического продукта.

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Introduction. Biological active substances usage in the form of natural vegetable extract in food, pharmaceutical, perfume and cosmetic industries cause the whole world researches' interest [1-3]. It is connected not only with final product increasing utility but its value growing as regional branding object [4-5].

In cosmetic industry there is a tendency of cosmetic products with natural ingredients providing a healing effect widespread usage. Natural extracts have a great advantage over synthetic ones that is conditioned by their various impacts on an organism, narrower range of contraindications and allergic effects.

By present time numerous facts proving the synthetic biocidal additives negative influence on organisms had been accumulated. So, metyl- and propylparabens cause allergic contact dermatitis [6]. The same data were obtained for other ethers of n – oxybenzoic acid [7, 8]. Benzyl alcohol and benzylparaben display allergiizing activity [9]. Benzoic acid and pyrrolidone carboxylic sodium salt provide contact reactions. Contacts dermatitis are caused by phenoxyethanol, also by biocides Euxyl K 400 mixture containing содержащей phenoxyethanol [10]. The identical phenomena can be observed for kathon CG, urea imidazolidinyl, clotrimazole, bronol, thimerosal, sorbic acid, propolis, quaternium, etc.

The high quality, competitive cosmetic compositions production is impossible without the relevant raw material base and natural nutrient, conserving, flavoring additives application. The most justified question solution is vegetative recourses usage for this purpose [11, 12].

Natural vegetative additives introduction into compositions is limited by poor investigation of extracts structure and property, combination with other ingredients. Consequently, the research in this field is topical. To a large extent it relates to medication from hanging birch (*Bétula péndula*) and black currant *Ríbes nígrum*) vegetative parts (containing a considerable set of biological active substances) taking into account the raw material volume and availability.

In recent years there is a great attention to the biocidal additives' safety problem. In this connection the research program contains the task of determining antibacterial, cytotoxic activity of water-alcohol currant leaves extracts, which, according to the received data, can be very useful in perfume and cosmetic compositions.

One more task is to investigate water alcohol currant and birch leaves extracts preserving effects depending on their concentration.

Materials and methods. Hanging birch (*Bétula péndula*) and black currant *Ríbes nígrum*) leaves plate naturally growing in the Krasnoyarsk region taiga were used as objects of investigation.

Leaves plates work pieces were made in the third decade of June – the first decade of July that is the period of this plant vegetative part maximum accumulation of biologically active substances.

After harvesting a plant leaves plates were dried in drying cabinets at the temperature of 40-50 °C, grinded and selected for fraction by size 5-10 mm investigation. Water-alcohol extraction was being done during an hour in the flask with reverse refrigerator at the temperature of solvent boiling and hydromodule 20. After cooling the obtained extract was filtered through paper filter (white strip).

As test-objects standard test cultures *Staphylococcus aureus, Escherichia coli, Shigella sonnei, Salmonella typhimurium, Candida albic* got from All-Russian microorganism collection and State Research Institute of standartization by Tarasevich were used.

The bacteria were grown in dense nutrient medium – meat-peptone agar (MPA). For this purpose the nutrient medium was sown by test-culture grass. Then the sterile disk of filter paper with 200 mkl of sterile investigated water-alcohol extract on its surface was put in the middle of Petri dish. The extract was gradually diffused into agar creating a painted zone around the disk. After that Petri dishes were incubated in the thermostat at the temperature of 27 °C. The consideration of investigated water-alcohol extract influence on bacteria growth and development was done 20 hours later. 200 mkl of sterile water were added to the filtered disk as a form of monitoring.

Antibacterial activity of the analyzed extracts was compared with benzoic acid activity which is presented in considerable amount in some plants, for example, in cranberry and lingonberry berries. While conducting the experiments the following concentrations were used, %: currant leaves extracts (pH 7,0) – 10,0; sodium benzoate (pH 5,5 и 7,0) – 0,3.

Water-alcohol currant and birth leaves extracts analysis on cytotoxicity was conducted on mice fibroblasts' test-culture – L929.

While experiments conducting the following preservatives' concentrations were used, %: birth leaves (water-alcohol extract) – 5,0 [8]; currant leaves (water-alcohol extracts) – 5,0; propolis (alcohol extract) – 5,0; sodium benzoate (powder) – 0,15; methyiparaben (powder) – as model biocidal system – 0,1.

The formed cell monolayer was treated by preservatives during 1 h and then in 24 h after medium changing to growth one the cells amount was counted.

All the definitions were made in triple repetition, after that the obtained values average arithmetic mean was calculated.

Results. The results of these experiments on water-alcohol birch (*Bétula péndula*) and currant leaves extracts (*Ríbes nígrum*) antibacterial activity are presented in the Table 1 [14 with addition].

Table 1. The preservatives influence on microbial test-cultures growth (average value in CFU/ml)

Preservative	Candida albicans	Staphylococcus aures	Escherichia coli	Shigella sonnei	Salmonella typhimurium
1	2	3	4	5	6
Currant leaves extracts	$1,0\times10^6$	$1,5\times10^7$	0	0	0
Birch leaves extract	$1,3\times10^6$	$1,8\times10^7$	0	0	0

1	2	3	4	5	6
Sodium benzoate (pH 7,0)	0	$2,6\times10^9$	$6,6\times10^8$	$1,5\times10^9$	$1,3\times10^9$
Monitoring (pH 7,0)	$1,0\times10^9$	$1,7\times10^9$	$5,6\times10^8$	$1,3\times10^9$	$3,6\times10^9$
Sodium benzoate (pH 5,5)	$6,4\times10^9$	0	0	$1,4\times10^5$	0
Monitoring (pH 5,5)	$3,8\times10^6$	$1,7\times10^4$	0	$4,3\times10^8$	$2,2\times10^7$

Table 2 – Ethyl alcohol influence on some microorganisms test-cultures development

The test-culture	Microorganisms amount, CFU/ml	
	monitoring	+ 10% ethyl alcohol
Salmonella typhimurium	$9,2\times10^8$	$1,1\times10^6$
Shigella sonnei	$1,0\times10^9$	$3,4\times10^7$
Staphylococcus aures	8×10^8	$5,5\times10^7$
Escherichia coli	2×10^8	$3,3\times10^6$

The Table 2 data witness that 10 %- ethyl alcohol oppresses cell growth in 1-2 orders of magnitude. On this basis, microorganisms' concentration decreasing from 3 to 9 orders of magnitude for alcohol currant leaves extract cannot be explained by only solvent influence. The observing oppression of their growth is caused by aggregate alcohol and extractive substances activity.

Sodium benzoate in acidic environment (in its turning to benzoic acid) for all test-cultures also possesses rather high biocide activity. However, at pH 7,0 it practically does not restrain cultures development. Only fungal culture *Candida albicans* is an exception, which growth is blocked even more effective than in acidic environment.

Taking into account that optimal value pH for cosmetic medications is near 7,0, currant leaves extract application can be recommended as the antibacterial preservative in combination with the sodium benzoate additive for preserving system fungicidal activity gaining.

The next research stage was the investigation of water-alcohol extracts cytotoxic activity.

During the experiment there were used in 2 times less preservatives concentrations than while biocide activity studying. As we have already shown in [15], such concentration declining was necessary because biological active substances water-alcohol extracts from birch (*Bétula péndula*) and black currant leaves (*Ríbes nígrum*) addition to nutrient medium in the same concentrations while investigating microorganisms growth inhibition led to practically total cell destruction.

The cells ratio in the monolayer (in comparison with monitoring) by the used preservatives activity is presented in the Figure 1.

Discussion. The obtained data prove that sodium benzoate and water-alcohol currant leaves extract can have the gentlest activity in relation to cell fiber cultures. Methyiparaben act the most negatively to cell culture L929.

To found out the cell system behavior dynamics in dependence on time and possible ethyl alcohol influence the additional experiment was conducted. During this experiment not only the remaining on the tablet after 1 hour treatment by biocidal additives and subsequent 24 hour growing in standard medium cells but the cells in the "flush" were evaluated. These values were summed in the alive and dead cells relative share calculation process.

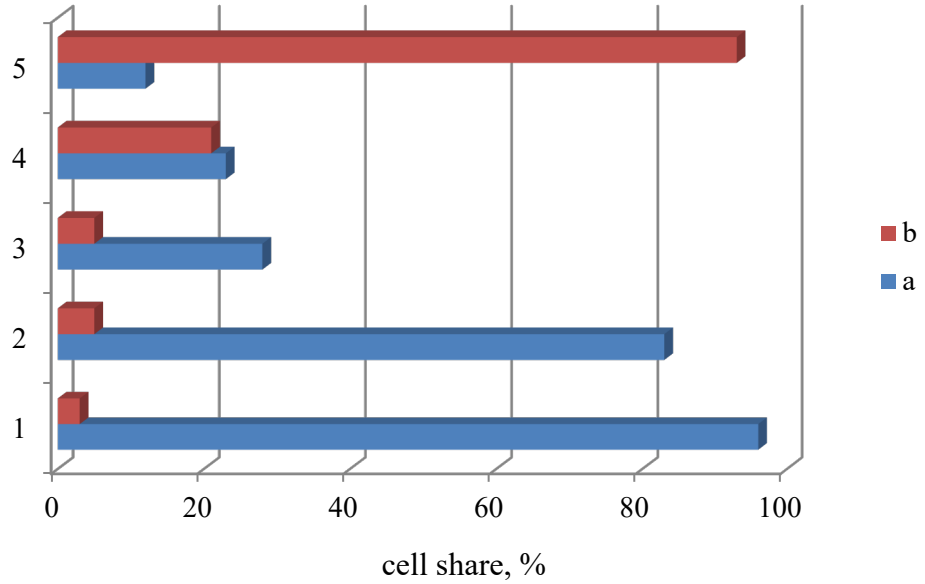

Figure 1. Cells amount, alive (a) and dead (b) in monolayer by preservatives activity on L929 (1 – sodium benzoate; 2 – currant leaves extracts; 3 – birth leaves extracts; 4 – propolis alcohol extract; 5 – methyiparaben)

The results obtained indicate that as opposed to the first experiment there is the birth leaves extract toxic action weakening. The results remained actually unchanged for currant leaves extract. Generally, the amount of sustainable cells determined directly after biocides one hour influence is decreasing as a result of further keeping in the growth medium.

The same result is being observed for dead cells that can be explained by their destruction (lysis) during the 24 hours exposition process. Only with the ethanol addition for the alive cells "the consequence positive effect" can be watched. This circumstance witnesses about the differences in the activity mechanisms of ethyl alcohol and birth and currant leaves extractive substances.

Conclusions. Birch (*Bétula péndula*) and black currant (*Ríbes nígrum*) leaves plates water-alcohol extracts antibacterial activity was performed.

It was established that black currant water-alcohol extract is the most effective. Birch leaves water-alcohol extract has less biocidal activity.

It was shown that 10 %- ethyl alcohol oppresses cell growth in 1-2 orders of magnitude. Consequently, microorganisms' concentration decreasing from 3 to 9 orders of magnitude is caused by aggregate alcohol and extractive substances activity.

It was found out that Sodium benzoate in neutral environment blocks fungal culture Candida albicans more effective than in acidic one what allow to recommend birch and black currant leaves as the antibacterial preservative in combination with the sodium benzoate additive for preserving system fungicidal activity gaining.

Low toxicity in relation to sodium benzoate cell fiber cultures and currant leaves extract was confirmed and some toxicity of birch leaves extract and propolis was verified. According to observation results currant leaves extract can be recommended as the least toxic biocidal natural additive; birch leaves extract can be used only in certain (minimal) concentrations.

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