

Influence of the Solvent on the Antioxidant Properties of Extracts

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Received: 20th August 2019, Accepted: 30th September 2019, Published: 31st October 2019

Abstract

The aim of this work was to study the content of biologically active compounds in aqueous and ethanol extracts of the roots and rhizomes of elecampane, meadow clover grass and sea buckthorn berries, as well as to determine the indicators of their antioxidant activity.

Water extracts were prepared in the form of two dosage forms recommended by the Pharmacopoeia of the Russian Federation - infusion and decoction, differing by the duration of the heat treatment.

In the extracts, the amount of antioxidant compounds such as phenolic compounds, flavonoids, carotenoids was estimated, and the content of chlorophylls was also determined in clover extracts. It is shown that 70 vol. % ethanol extracts all detectable compounds more efficiently than water. The largest amount of biologically active substances (except chlorophylls) was extracted from sea buckthorn berries.

The restorative power and antiradical activity of the extracts were investigated. It was shown that in the case of each plant separately, ethanol extracts showed a higher reducing power and antiradical activity compared to water. However, when comparing plant extracts with each other, it was found that both ethanol and even water extracts of sea buckthorn berries in these indicators prevailed over ethanol extracts of elecampane and clover.

Keywords

Elecampane, Meadow Clover, Sea Buckthorn, Phenolic Compounds, Flavonoids, Carotenoids, Chlorophylls, Antioxidants

Introduction

Protection of food from oxidation is an urgent task [1]. As a source of antioxidants can be used medicinal plants, which are used in medicine as prophylactic agents, as well as to facilitate the course of diseases. These plants include elecampane, clover and sea buckthorn, which are widespread in the Russian Federation.

Elecampane (*Inula L.*) is a perennial medicinal plant from the Asteraceae family. The roots of elecampane have a specific sweetish smell and a bitter burning taste. In the food industry, elecampane is used in the manufacture of confectionery and drinks. The essential oil contained in the roots and rhizomes is used to flavor fish and culinary products. In medicine, elecampane preparations are used as anti-inflammatory drugs in the treatment of pulmonary and intestinal diseases. Biologically active substances, elecampane give it a diuretic, choleric, expectorant, antimicrobial, anti-inflammatory and anthelmintic action [2].

The main groups of active substances of elecampane are sesquiterpene lactones, hydroxycinnamic and hydroxybenzoic acids, flavonoids [2].

Meadow clover (*Trifolium pratense L.*) is a perennial herbaceous plant from the Fabaceae family, a good honey plant. Clover flowers and leaves contain a large number of biologically active substances: essential oils, tannins, glycosides trifolin and isotrifolin, isoflavones, flavonoids and vitamins [3].

Sea buckthorn (*Hippóphaë*) is a thorny shrub of the Elaeagnaceae family. Sea buckthorn berries are a source of vitamins C, E and group B, carotenoids, flavonoids, triterpenic acids, amino acids, micro and macro elements [4].

Sea buckthorn oil consists of seed oil containing ω -3 and ω -6 essential fatty acids, and berry pulp oil in which a high content of ω -7 palmitoleic acid is found. These fatty acids accelerate skin regeneration during burns, and contribute to the healing of ulcers of the mucous membranes of the body [4].

The aim of this work was to determine the content of biologically active compounds in aqueous and ethanol extracts of the roots and rhizomes of elecampane, meadow clover grass and sea buckthorn fruits and to study their antioxidant properties.

Methods

The roots and rhizomes of elecampane (*Inula L.*) (Krasnogorsklexredstvo JSC, Krasnogorsk, Moscow Region), meadow clover grass (*Trifolium pratense L.*) (Lekra Set LLC, Altai Territory, Barnaul) were used in the work and

dried berries of sea buckthorn (*Hippophae rhamnoides* L.) (LLC "Natural Products", St. Petersburg). Before extraction, the raw materials were ground in a laboratory mill.

To prepare water extracts, a weighed portion of the crushed plant material was poured into a flask, filled with distilled water in a ratio of 1:10, and the flask was placed in a boiling water bath for 15 minutes (infusion) and 30 minutes (decoction), constantly stirring. After removing the flasks from the water bath, the infusion was kept at room temperature for 45 minutes, and the broth for 10 minutes [5].

To prepare ethanol extracts, a weighed portion of crushed plant material was poured with a boiling solution of ethyl alcohol at a concentration of 70 vol. % in a ratio of 1:10 and extraction was carried out at a temperature of 70 °C for an hour with constant stirring. The prepared aqueous and ethanol extracts were filtered through a filter with a pore size of 0.45 µm.

Spectrophotometric studies were performed on an SF-2000 spectrophotometer (OKB Spectrum LLC, St. Petersburg). The total number of phenolic compounds was determined according to the method of Folin and Ciocalteu in the modification of Singleton V.L. et al. [6]. A calibration graph was built on the absorption of gallic acid solutions. The results were expressed in mg · gallic acid equivalents / L.

The total amount of flavonoids in the extracts was determined according to the method proposed by Chang, C.-C. et al. [7]. A calibration graph was built on the absorption of quercetin solutions. The results were expressed in µg equivalents of quercetin / ml.

To determine the content of carotenoids in extracts of elecampane roots and sea buckthorn fruits, they were spectrophotometrically measured in a cuvette with a layer thickness of 10 mm at 450 nm (maximum absorption of carotenoids) relative to 95% ethanol. The concentration of carotenoids was determined by the formula, mg / ml:

$$C_{car} = \frac{A \cdot V_1}{A^{1\%}} \cdot C^{1\%},$$

where A – absorption of the dissolved sample;

V_1 – dilution factor;

$A^{1\%}$ – absorption of a 1% solution of β -carotene ($A^{1\%} = 2620$);

$C^{1\%}$ – concentration of 1% solution (10 mg / ml) [8].

Due to the fact that chlorophylls interfere with the spectrophotometric determination of carotenoids, the content of chlorophylls and carotenoids in clover grass extracts was determined by the method of Lichtenthaler H.K. and Buschmann C. [9]. The extract was spectrophotometrically measured in a cuvette with a layer thickness of 10 mm at a wavelength of 470 nm (maximum absorption of carotenoids), at 648.6 nm (maximum absorption of chlorophyll b) and 664 nm (maximum absorption of chlorophyll a) relative to 95% ethanol. The concentration of chlorophyll a, chlorophyll b and carotenoids in the samples was determined by the formulas, µg / ml:

$$C_a = 13,36 \cdot A_{664,1} - 5,19 \cdot A_{648,6}$$

$$C_b = 27,43 \cdot A_{648,6} - 8,12 \cdot A_{664,1}$$

$$C_{car} = \frac{(1000 \cdot A_{470} - 2,13 \cdot C_a - 97,64 \cdot C_b)}{209}$$

where A_{470} , $A_{648,6}$, $A_{664,1}$ – absorption of the diluted sample at appropriate wavelengths.

Reducing power of the extracts was determined by the ferricyanide method according to Lertittikul W. et al. [10]. The optical density of the reaction mixture was measured at 700 nm. The reducing force was expressed relative to the control — 0.01% ascorbic acid solution, the reducing force of which was taken as 100%.

Antiradical activity was determined by a modified method based on a decrease in the optical density of a solution of 2,2-diphenyl-1-picrylhydrazyl free stable radical (DPPH) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in the presence of antioxidants [11].

The percentage of inhibition of DPPH radicals was determined by the formula:

$$\% \text{ inhibition of DPPH} = [(D_K - D_O) / D_K] \cdot 100,$$

where D_O – optical density of the experimental sample;

D_K – optical density in the absence of antiradical substances (control).

At the same time, the percent inhibition of DPPH was determined for known concentrations of Trolox (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and a calibration curve was constructed. The antiradical activity of the extracts was expressed in µmole Trolox-Equivalent.

All experiments were performed in triplicate, after which the obtained data were processed by statistical methods using Microsoft Excel software. Results are presented as mean ± standard deviation (mean ± SD).

Results and Discussion

The chemical composition of the extracts depends on the extraction method, type and polarity of the extractant, the method of preliminary preparation of raw materials and the conditions for the extraction. Solutions of ethanol,

methanol, acetone and water are used to extract flavonoids and phenolic acids. As extractants in our work, we used distilled water and a solution of ethyl alcohol with a concentration of 70 vol. %

Phenolic compounds are ubiquitous secondary metabolites of plants, they have a wide range of biological properties: antioxidant, antiradical, anti-inflammatory, antimicrobial, anticancer, antiviral, etc.

Plant phenols have a diverse structure, they differ in the number of aromatic rings, the location and nature of the substituents. The main groups of phenolic compounds are: flavonoids, phenolic acids, tannins, stilbens and lignans.

The content of phenolic compounds in the studied extracts is presented in Figure 1.

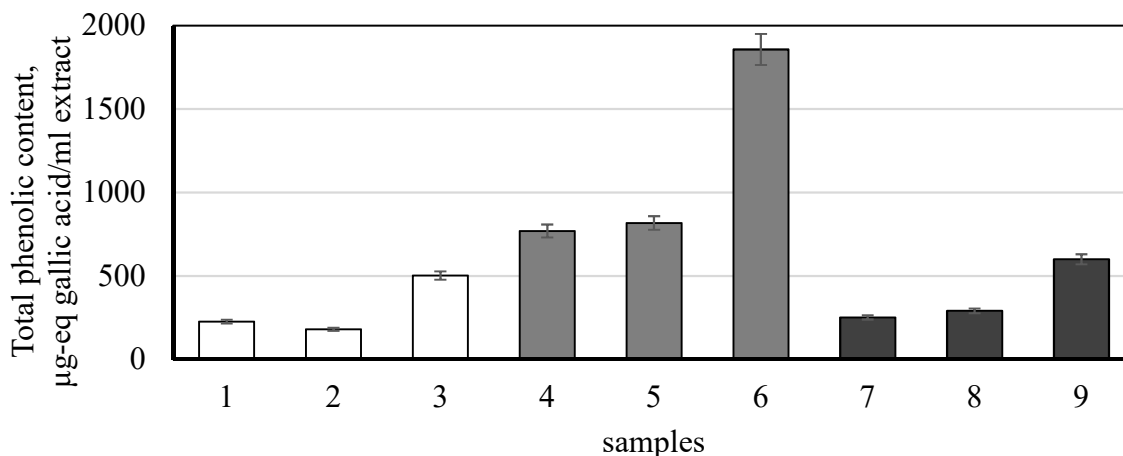


Figure 1: The Total Content of Phenolic Compounds in the Extracts

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass; 4 - water infusion of sea buckthorn berries; 5 - water decoction of sea buckthorn berries; 6 - ethanol extract of sea buckthorn berries; 7 - water infusion of the roots of elecampane; 8 - water decoction of the roots of elecampane; 9 - ethanol extract of the roots of elecampane)

It was found that ethanol is the preferred extractant for phenolic compounds, the content of which in alcoholic extracts was almost two times their amount in aqueous extracts for all types of plant materials. This is due to the fact that phenolic compounds such as hydroxycinnamic and hydroxybenzoic acids, which are widespread in the plant world, are better soluble in alcohols than in water [12]. The greatest amount of phenolic compounds was extracted with ethanol from the fruits of sea buckthorn.

Currently, more than 8,000 phenolic compounds have been identified, of which more than 4,000 are various flavonoids. They are low molecular weight compounds of 15 carbon atoms forming a structure containing two aromatic rings. A variety of substituents are attached to the basic structure in certain places, which explains the great variety of flavonoids.

Flavonoids are strong antioxidants, they exhibit reducing activity, are hydrogen donors, singlet oxygen quenchers and can exhibit chelating properties.

The flavonoid content in the extracts is shown in Figure 2.

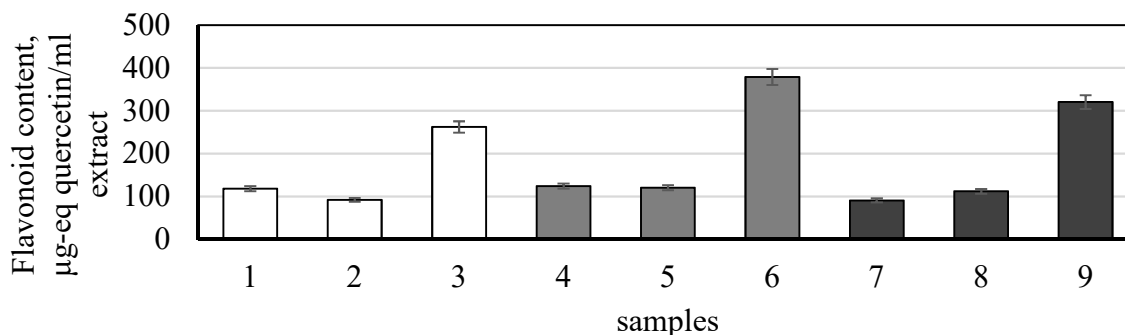


Figure 2: Flavonoid Content in Extracts

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass; 4 - water infusion of sea buckthorn berries; 5 - water decoction of sea buckthorn berries; 6 - ethanol extract of sea buckthorn berries; 7 - water infusion of the roots of elecampane; 8 - water decoction of the roots of elecampane; 9 - ethanol extract of the roots of elecampane)

As can be seen from Figure 2, flavonoids are better extracted from plant materials with ethanol. The content of flavonoids in ethanol extracts exceeded this indicator in aqueous extracts by 2-3 times.

In [13], it was found that the solubility of flavonoids increases with increasing molar fraction of alcohols in a mixture with water.

In water extracts, the amount of flavonoids was almost the same for all types of raw materials, which is probably due to their limited solubility in water. A comparison of ethanol extracts shows that more flavonoids were extracted from sea buckthorn than from elecampane and clover, which characterizes sea buckthorn berries as a more promising source of flavonoids.

Biologically active compounds that are widespread in the plant world are carotenoids - yellow-orange fat-soluble pigments. By their chemical nature, they belong to isoprenoid compounds. A large number of conjugated double bonds in carotenoid molecules determines their antioxidant properties, in particular, gives them the ability to quench singlet oxygen and free radicals. The content of carotenoids in the extracts is shown in Figure 3.

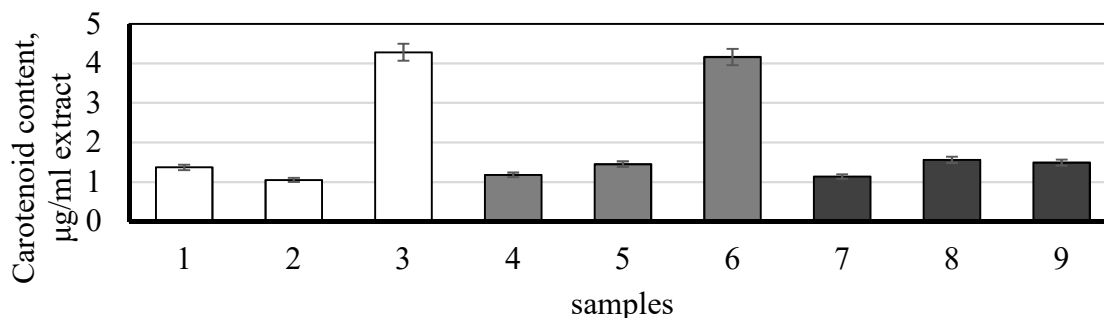


Figure 3: The Content of Carotenoids in Extracts

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass; 4 - water infusion of sea buckthorn berries; 5 - water decoction of sea buckthorn berries; 6 - ethanol extract of sea buckthorn berries; 7 - water infusion of the roots of elecampane; 8 - water decoction of the roots of elecampane; 9 - ethanol extract of the roots of elecampane)

From the data in Figure 3 it can be seen that clover and sea buckthorn contain almost three times as many carotenoids as compared to elecampane. A more effective extractant for extracting carotenoids, as in previous cases, turned out to be 70 vol. % ethanol.

In contrast to elecampane and sea buckthorn, clover grass also contains chlorophylls with antioxidant properties, which are presumably due to the nature of the metal ion in the porphyrin ring and are manifested in the protection of hydroperoxides from decomposition [14]. The content of chlorophyll in clover herb extracts is shown in Figure 4.

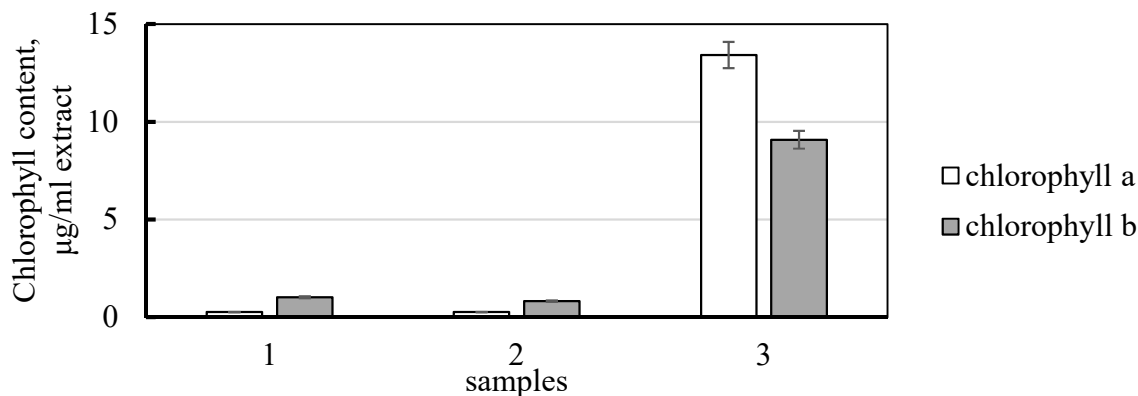


Figure 4: The Content of Chlorophyll in Extracts of Clover Grass

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass)

As can be seen from Figure 4, chlorophylls practically did not dissolve in water and were found in significant quantities only in the ethanol extract, in which chlorophyll a prevailed.

Next, we determined the reducing power of the extracts. The results are presented in Figure 5.

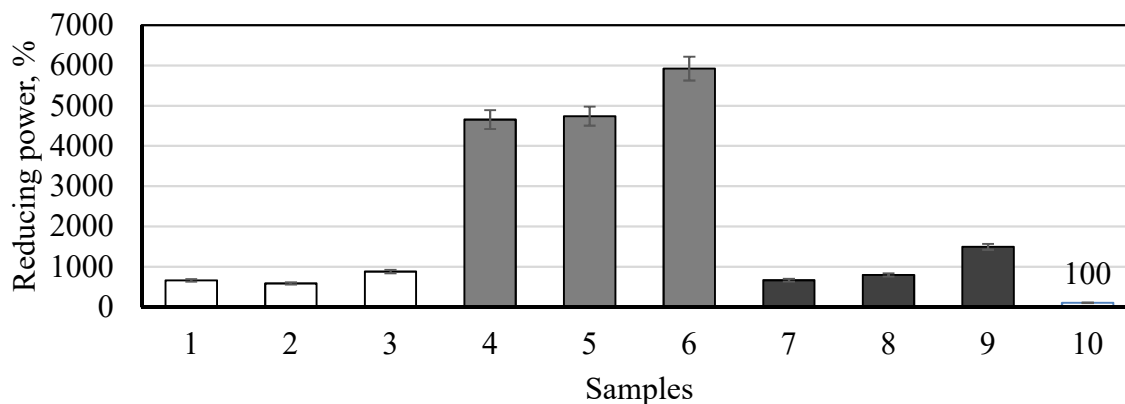


Figure 5: The Restorative Power of the Extracts Relative to a 0.01% Solution of Ascorbic Acid

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass; 4 - water infusion of sea buckthorn berries; 5 - water decoction of sea buckthorn berries; 6 - ethanol extract of sea buckthorn berries; 7 - water infusion of the roots of elecampane; 8 - water decoction of the roots of elecampane; 9 - ethanol extract of the roots of elecampane; 10 - 0.01% solution of ascorbic acid)

The data obtained show that the degree of influence of the nature of the extractant on the reducing power of the extracts fluctuated greatly, from insignificant in clover to significant in elecampane. A similar effect indicates a different sensitivity of the antioxidant compounds of the studied plants to the polarity of the solvent. The greatest restorative power was shown by sea buckthorn extracts.

The higher reducing power of extracts of sea buckthorn and ethanol extract of elecampane corresponds to high levels of phenolic compounds and partly flavonoids. However, on the example of water extracts of sea buckthorn, the content of flavonoids in which was lower than in the ethanol extracts of elecampane and clover, and the reducing force, on the contrary, is greater, it can be seen that other components also contribute to the antioxidant properties. It can be ascorbic acid and tocopherols, also found in sea buckthorn berries [4]. At the same time, carotenoids and chlorophylls have little effect on the reducing power of extracts; this is probably due to the lipophilic nature of these compounds, which does not allow them to exhibit antioxidant properties in the aqueous medium of the ferricyanide reaction.

Many biologically active compounds exhibit the ability to quench free radicals. We have studied the influence of the nature of the solvent on the antiradical activity of the extracts. The results are presented in Figure 6.

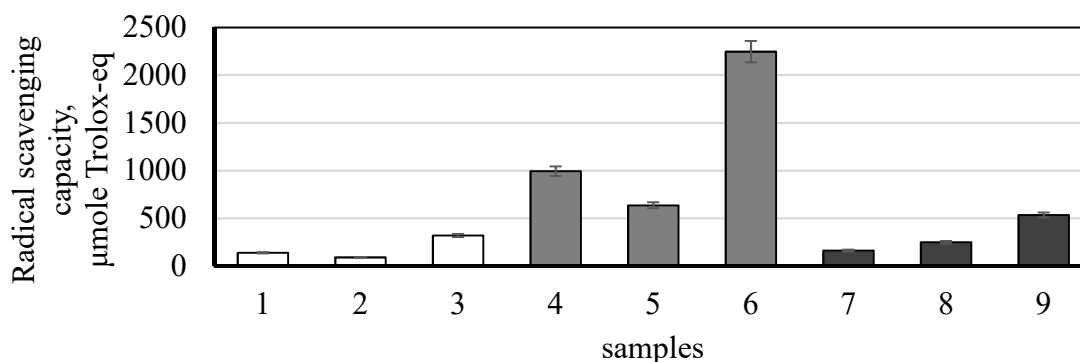


Figure 6 : Antiradical Activity of Extracts

(1 - water extract of clover grass; 2 - water decoction of clover grass; 3 - ethanol extract of clover grass; 4 - water infusion of sea buckthorn berries; 5 - water decoction of sea buckthorn berries; 6 - ethanol extract of sea buckthorn berries; 7 - water infusion of the roots of elecampane; 8 - water decoction of the roots of elecampane; 9 - ethanol extract of the roots of elecampane)

Due to the fact that in this method the reaction proceeds in a medium containing ethanol, an increase in the contribution of lipophilic components to the antioxidant defense was observed. Ethanol extracts had the highest antiradical activity within the same plant. It was two times higher than that of aqueous extracts.

In general, the extracts of sea buckthorn were superior to the extracts of elecampane and clover, in anti-radical activity, regardless of the nature of the solvent.

It was noted that with an increase in the duration of extraction, the antiradical activity of water extracts of elecampane increases, while clover and sea buckthorn, on the contrary, decreases, which indicates different thermal stability of their antiradical components.

Thus, in the preparation of water extracts of elecampane, a long (30 min) regimen for extracting biologically active compounds can be recommended, and for clover and sea buckthorn a short one (15 min).

The more pronounced antiradical activity of ethanol extracts, compared with aqueous extracts, corresponds, as shown above, to a high content of antioxidants in them and is probably associated with a low polarity of antiradical compounds, due to which they dissolve better in 70 vol. % ethanol.

Summary

For the extraction of biologically active components of elecampane, ethanol is the preferred solvent, since it extracts more antiradical compounds from the raw material compared to water.

The preferred method of preparing water extracts is an infusion (15 minutes in a boiling water bath), since many active substances have low thermal stability and their prolonged heating is undesirable, as evidenced by a decrease in the antiradical activity of clover and sea buckthorn extracts with an increase in the duration of extraction.

Conclusions

Thus, the data obtained show ethanol extracts to be more promising sources of antioxidants for the food industry than water. However, water extracts are also necessary, as they can be used to produce products for children and people who have contraindications for using alcohol.

Acknowledgements

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

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