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# PREPARATION OF COMPONENTS OF ULTRASONIC EXTRACT OF GINKGO BILOBA, PHYSICO-CHEMICAL AND PHARMACOLOGICAL ANALYSIS AND MOLECULAR DESIGN

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**ABSTRACT** — The main task arising during the processing of medicinal plant raw materials is the intensification of extraction processes in order to maximize the extraction of groups of biologically active substances. It is of interest to obtain an extract and conduct molecular modeling in order to predict the biological activity of the components of the ginkgo biloba phytocomplex. The range of dosage forms obtained on the basis of dry extract is small, despite the wide range of pharmacological activity. THE AIM OF THE STUDY is to optimize the method of obtaining alcohol extract, with the possibility of including it in various dosage forms. PHYSICO-CHEMICAL ANALYSIS: Confirmation of the alleged pharmacological activity by the method of molecular modeling. MATERIALS AND METHODS: Ultrasonic extraction, spectrophotometry in the ultraviolet region. Pharmacological studies by modeling cerebral ischemia using simultaneous bilateral occlusion of the common carotid arteries. Changing the speed of cerebral blood flow by Dopplerography. RESULTS AND DISCUSSION. Liquid extract of ginkgo bilobar leaves was obtained by ultrasonic extraction. The calculation of the electronic structure and quantum-chemical descriptors of ginkgolide B. was carried out. The assessment of specific pharmacological activity was carried out. CONCLUSION: The ultrasonic method of extraction of ginkgo biloba leaves was applied. The predicted biological activity of the obtained phytocomplex was confirmed by methods of molecular modeling and experimentally.

**KEYWORDS** — ultrasonic extraction, UV spectrometry, ginkgo biloba, ginkgolide B, flavonoids, molecular modeling.

## INTRODUCTION

One of the main tasks that arise in the processing of medicinal plant raw materials is to intensify the extraction of groups of biologically active substances to increase the yield of extractive substances. A large number of studies have been devoted to the study of the pharmacological properties of preparations based on plants of the birch family growing on the territory of Russia, however, the products of processing of these plants still have a significant medical potential. Another of the phytoobjects, the raw material base of which is successfully developing, is Ginkgo biloba [1]. The range of known types of biological action is very wide [2]. However, the range of medicines obtained on the basis of dry Ginkgo biloba extract has few positions. Dry extract from the leaves of Ginkgo (Ginkgo biloba L.), is widely used in the manufacture of medicinal preparations .It is produced by extraction with a established optimal extractant followed by purification with organic solvents (chloroform, acetone, ethyl alcohol).This control is subject to pharmaceutical substances and excipients, as well as drugs, regardless of the method of their use, if organic solvents are used in their preparation or purification.In this regard, the rejection of traditional chloroform extraction and the transition to high-performance ultrasonic extraction with ethyl alcohol is justified. However, the use of US does not always lead to positive results. Sometimes in plant cells, wall rupture and a change in the physicochemical properties of some components occur. Therefore, in each individual case, it is necessary to carefully study the effect of ultrasound on specific substances and under specific conditions .

Substances of different chemical groups, characterized by diverse pharmacotherapeutic activity, are isolated from Ginkgo leaves. They are mainly represented by diterpenes, sesquiterpenes and flavonoids. In this regard, the range of application of this object is very wide: it is a therapy of cerebral ischemia and chronic cerebrovascular insufficiency, normalization of microcirculation and providing a powerful general antioxidant effect [2-4]. The positive effect of G. biloba extract on the rheological properties of blood is also known. However, the number of dosage forms of

ginkgo is small. Medicinal products of ginkgo leaves, which include dry extract, are widely used (Tanakan, Bilobil, Memoplant, Ginkor Fort, Ginkor Gel, etc.).Therefore, the creation of various dosage forms based on these plant objects with a sufficient raw material base is quite an urgent task. But obtaining any phytocomposition of varying degrees of technological complexity always begins with the extraction process; it is the central and dominant process in the technology of all, without exception, plant-based preparations. [5].

Materials and Methods

The use of ultrasound is much more effective in comparison with mixing, as well as the use of high temperatures and pressures. With the help of ultrasound, almost any substance can be extracted from plant materials [6]. The use of ultrasound not only increases the speed of the process, but also provides an increase in the yield of the target product in comparison with other extraction methods [7,8].

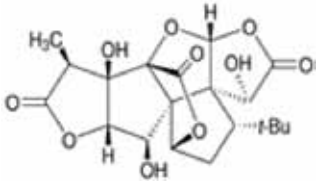
A ginkgo biloba leaf was poured into an ultrasonic extractor NO-230.00P, filled with 70% ethyl alcohol, and thoroughly mixed with a built-in stirrer to remove air. The lid of the extractor was closed and extraction was carried out, cooling the system with cold water supplied to the casing of the apparatus. Thus, the extraction lasted 120 minutes.Next, the optical density of the samples obtained during ultrasound extraction of G. biloba extract was studied by UV spectrometry on an Ecros PE-5400UF spectrophotometer (OOO Ecros-Analytics, Russia, St. Petersburg) at the maximum light absorption at a wavelength 415 nm in a cell with a layer thickness of 10 mm. 2 ml of the sample was placed in a volumetric flask with a capacity of 50 ml and brought up to 70% with ethyl alcohol (solution A). 2 ml of solution A was placed in a volumetric flask with a capacity of 25 ml, 2 ml of a 2% solution of aluminum chloride in 96% ethanol and 0.1 ml of a solution of acetic acid diluted were added. The volume of the solution was adjusted to the mark with the same alcohol (solution B) and left for 40 minutes. The optical density of the solution was measured at the maximum light absorption at a wavelength of 415 nm in a cell with a layer thickness of 10 mm. As a comparison solution, a solution consisting of 2 ml of extraction, 0.1 ml of a solution of diluted acetic acid and 70% ethyl alcohol added to the mark in a 25 ml volumetric flask was used. In parallel, the optical density ofthe reference standard (RS) of rutin solution prepared analogously to the test solution was measured [5].

Molecular Docking

The calculation of the electronic structure and quantum chemical descriptors of ginkgolide B was the second stage of theoretical research. Molecular descriptors obtained using the publicly available programs MOPAC, DRAGON and GAMESS were used. The assessment of the specific pharmacological activity of the developed dosage forms of Ginkgo biloba was performed on 24 male rabbits of the "California" breed weighing 2.5-3.0 kg, obtained from the laboratory animal nursery "Rappolovo" (Leningrad Region). Before the study, the animals were kept in quarantine conditions for 14 days. During the direct conduct of the experiment, the rabbits were kept in a vivarium under controlled climatic conditions: at an air temperature of 20±2°C, a relative humidity of 60±5% and a 12-hour change of the daily cycle in mesh metal cages equipped with a drip drinker and a feed supply tank. The number of species in one cell was four. The animals' access to food and water was not restricted. The study of the cerebrotropic activity of the developed dosage forms of Ginkgo biloba was carried out on a model of bilateral occlusion of the common carotid arteries. The standardized extract of Ginkgo biloba (EGB 761), obtained from Hunan Warrant Pharmaceuticals (PRC), was used as a comparison drug. The studied dosage forms and the reference drug were administered in a therapeutic mode after modeling brain ischemia once a day for 3 weeks. The comparison drug was administered orally at a dose of 35 mg / kg [2], the analyzed dosage forms (drops, sodium alginate-based gel and chitosan-based gel) were administered intranasally at a dose equivalent to that of the comparison drug. During the study, changes in neurological deficits were determined according to the McGraw scale (the initial indicator, as well as that on the 3rd, 7th, 14th and 21st days of the experiment), the average systolic velocity of cerebral blood flow (on the 3rd, 7th, 14th and 21st days of the experiment) and the pro/antioxidant balance in the hippocampus (on the 21st day of the study) [9,10].

Results and Discussion

The calculation of the electronic structure and quantum chemical descriptors of ginkgolide B was carried out. This calculation was performed by us for the first time, data on the analysis of such structures were found by us in only one report by Chinese scientists [11], however, exact quantum chemical calculations were not performed. The calculation was carried out in the HyperChem 8.0 chemical package (license number HC80SA-4-1BBF6). Visualization of the structure demonstrates its steric hindrance.



To determine the chemically active part of the molecule, we first performed the calculation of the energy of the boundary orbitals (namely, the upper occupied molecular orbital) by the AM1 method and the subsequent computer simulation (electron density is shown in blue and green).(Fig 1.)An analysis of the image shows that despite the presence of several potential active centers, the ginkgolide B molecule has only one active center, and it is apparently responsible for the interaction of the molecule with platelet activating factor (PAF)

(Fig. 2. ).The conducted complex of studies allowed us to establish that in conditions of experimental cerebral ischemia modeled by simultaneous occlusion of the common carotid arteries in large laboratory animals (rabbits), the use of the obtained extract contributed to a decrease in the severity of neurological deficit, restoration of cerebral hemodynamics and pro/antioxidant balance in the hippocampus.

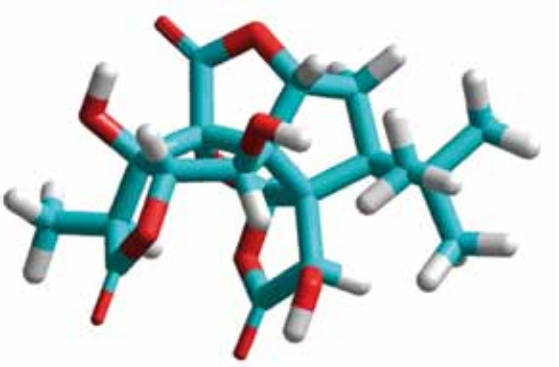


***Fig. 1.*** *Formula and three-dimensional structure of ginkolide B*

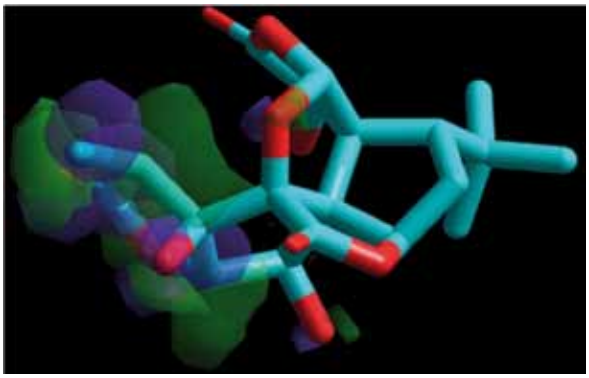


***Fig. 2.*** *Computer simulation of the highest occupied molecular orbital of ginkgolide B*

## CONCLUSION

Thus, based on the theoretical results and experimental data obtained, it can be assumed that the ginkgo biloba extract obtained by ultrasound extraction may be a promising means of correcting chronic cerebral circulatory disorders with an antioxidant mechanism of action and therapeutic potential superior to the existing, standardized ginkgo biloba extract — EGB 761.

## REFERENCES


1. **Shtrygol, S. Y. Shtrygol D V., Nazarenko M.E.** Standardized extract of ginkgo biloba: components, mechanisms of action, pharmacological effects, application. 2005 – No 4.
2. Copyright certificate NUMBER SU 1286232A1B01D11/02. Method of extraction from a solid. A. A. Dolinsky, V. N. Mudrikov, R. N. Korchinsky (USSR). – No. 3936068/31–26; Publ. 08.08.1985, B. I. No. 22.
3. **Kurkina A.V., Dubishchev A.V., Korchagina D. V., Bulankin D. G., Zagoskina N. V.** Ginkgo double-lobed – a promising source of import substitution. nootropic drugs. Traditional medicine. 2012. No. 5. pp. 261–265.
4. Development and research of phytoextracts containing flavonoids. Ogai M.A., Kovtun E.V., Chakhirova A.A., Samoryadova A.B., Bogatyreva Z.N. Scientific result. Medicine and pharmacy. 2018. Vol. 4. No. 2. pp. 90–103.
5. **Sasakia K.** Chemistry and biological activity of ginkgo biloba. – Hokkaido, 2007. – 177 p.
6. **Novitsky B. G.** Application of acoustic vibrations in chemical and technological processes. Moscow: Chemistry, 1983
7. **Ginstling A.M., Baram A.A.** Ultrasound in the processes of chemical technology. Leningrad: GOSKHIMIZDAT, 1960
8. **Gershal D. A., Friedman V. M.** Ultrasonic equipment. Moscow: Energiya, 1967.
9. The study of the neurotropic activity of drugs based on the leaves of ginkgo biloba // Korchagina D.V., et al. // Fundamental research. – 2013. – No. 10 – 4. – pp. 812–815
10. **Lee D., Miwa U, Jung H, Ikeda SI, Katada U, Tsubota K, Kurihara T.** A mouse model of ischemic retinal damage caused by temporary bilateral occlusion of the common carotid artery. J Compared to Exp. 2020 on November 12;(165). doi: 10.3791/61865. PMID: 33252109.
11. **Zhu B1, Chen G, Hu L, Lo X, Gui S, Lo S, Pua SM, Chen K, Jiang H.** QSAR analysis on ginkgolides and their analogues using CoMFA, COMSIA and HQSAR. Bioorg Med Chem.2005 January 17; 13(2): 313–22.