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# *Inula helenium l.* Root Extract in Sunflower Oil: Determination of its Content of Water-soluble Vitamins and Immunity-promoting Effect

**Ozoda Gaibullayeva[1]*, Akmal Islomov[2], Dilnoza Abdugafurova[2], Boynazar Elmurodov[3], Baxodir Mirsalixov[3], Lazizbek Mahmudov[2], Izzatullo Adullaev[2], Ko'zijon Baratov[2], Sirojiddin Omonturdiev[2] and Sitora Sa'dullayeva[4]**

[1]Department of Biology, Navoi State Pedagogical Institute, Uzbekistan, Navoi.
[2]Department of Pharmacol S.Sadykov Institute of Bioorganic Chemistry of the Academy Sciences of Uzbekistan, Tashkent.
[3]Department of Physio-Chemical Tashkent State Transport University postal code: 100060. Tashkent city, Odilhozhayev street, 1 house.
[4]Samarkand State University named after Sharof Rashidov.
*Corresponding Author E-mail: ozodagaybullayeva28081992@gmail.com

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**The search for new biologically active products derived from local plant resources remains an important area of scientific research. This study focused on the chemical composition and immunomodulatory potential of the oil extract from *Inula helenium L.*, a widely distributed perennial herb of the Asteraceae family. The analysis using (HPLC) identified several key water-soluble vitamins in the extract, with concentrations per gram as follows: B1 (0.0319 mg), B2 (0.9509 mg), B6 (0.6214 mg), B9 (1.8689 mg), and Vitamin C (0.2499 mg). The immunotropic activity of the oil extract was evaluated through a series of experiments in mice. The extract, administered intragastrically at 200 mg/kg, significantly increased macrophage counts in the peritoneal fluid, indicating a 1.6-fold rise compared to control. It also enhanced the cellularity of primary and secondary immune organs, including the thymus and spleen, with thymocyte and splenocyte counts rising by 1.3- and 1.7-fold, respectively, compared to healthy animals. Additionally, lymph node cellular content and mass increased, reflecting the extract's positive effect on peripheral immunity. Notably, the extract boosted bone marrow cellularity with a promotion index (PI) of 1.7, surpassing that of reference immunostimulant drugs. These results indicate that the oil extract of *Inula helenium* L. has notable immunostimulatory effects and may be useful in creating immune-boosting therapies. Additional research is needed to investigate the molecular mechanisms involved and assess the clinical effectiveness of this extract.**

**Keywords:** Alantolactone, anthelmintic, antiseptic, benzoic acid, carbohydrates, dihydroalantolactone, expectorant, *Inula helenium L.*, *Inula grandis*, inulin, and isoalantolactone are key bioactive compounds contributing to the medicinal properties of these plants.

Worldwide, extensive research is underway to create new, effective products from local resources. In this context, *Inula helenium L.*, a widely distributed perennial herb of the *Asteraceae* (Compositae) family, is particularly notable. This plant reaches a height of 110–160

cm, featuring upright, branched, and hairy stems. The basal leaves are large (up to 50 cm in length), long-stemmed, and either elliptical or elongated-ovate with pointed tips that narrow toward the base. Stem leaves are smaller, elongated-ovate, and decrease in size toward the top of the stem, with serrated edges. The upper leaf surface has sparse stiff hairs, while the underside is soft and densely hairy[1]. The upper stem leaves are sessile, while the lower leaves are alternately arranged along the stem with short petioles. The flowers are golden-yellow, grouped into heads that form a corymb or raceme at the top of the stems and branches. The involucral bracts are arranged in a tiled pattern, with ovate, recurved bracts covered in fine hairs. The marginal flowers are yellow and ligulate, while the central flowers are also yellow, tubular, and feature pappus bristles. The flowers include a calyx that develops into bristles, a corolla, five stamens, and a unilocular, inferior ovary. The fruit is an elongated, four-edged, brown or dark-brown achene[2].

The flowering period lasts from May to September, with the fruits maturing between July and October. The above-ground part of *Inula helenium L.* is shown in Figure 1.

The chemical makeup of *Inula helenium* includes 1-3% essential oil, up to 44% inulin and other carbohydrates, trace amounts of alkaloids, acetic and benzoic acids, as well as saponins. The essential oil forms a quickly solidifying crystalline substance with a characteristic aroma and flavor. Its crystalline element, known as helenin, is a blend of three selinan-type sesquiterpene lactones (alantolactone, isoalantolactone, and dihydroalantolactone). Besides helenin, the essential oil contains minor amounts of alantol and proazulene. The aerial parts of the plant have up to 3% essential oil, while the leaves contain a bitter compound called alantopicrin[3].

Inula helenium L. is utilized in medicine as an expectorant and for managing gastrointestinal issues. Its essential oil has antiseptic, anthelmintic, and anti-inflammatory properties, with the anthelmintic effects linked to alantolactones, which act similarly to santonin4. Medicinal extracts from the rhizome and roots, such as Allanton, provide anti-inflammatory, vascular-strengthening, and antiseptic benefits, aiding in the healing of gastric ulcers. Additionally, the plant has shown potential cardiovascular advantages, including vasorelaxant and cardioprotective effects, making it of particular interest in cardiovascular research5.

## MATERIALS AND METHODS

### Chemicals

Sunflower oil: Food-grade, used as the extraction solvent.

Ethanol (96%): Analytical grade, used for primary extraction (Sigma-Aldrich).

NaCl (0.9%): Isotonic saline solution, used for oral administration and preparation of cell suspensions (Sigma-Aldrich)[5].

Acetic acid (3%): Analytical grade, used for nucleated cell counting.

Immunal: Commercial immune stimulant containing 80 mg dry juice of *Echinacea purpurea* (Sandoz, Slovenia).

Red May: Herbal product containing St. John's wort (12 g), nettle leaves (2 g), rose petals (1 g), licorice root (1.6 g), creeping thyme herb (200 mg), lemon balm leaves (200 mg), and sea buckthorn oil (1.6 mg) (Phytoleum LLC, Kazakhstan).

Vitamins (Water-Soluble Standards): Vitamin B1, B2, B6, and C, analytical grade (1 mg/ml stock solutions prepared in 40% ethanol, Sigma-Aldrich).

Acetonitrile (HPLC grade): Used as the mobile phase in HPLC analysis (Merck)[6].

Acetate buffer: pH 5.0, used sodium acetate and acetic acid for HPLC separation.

HPLC System: Agilent-1200 with Eclipse XDB C18 column (5 µm, 4.6 × 250 mm) and Diode Array Detector (DAD).

### Method for Obtaining the Oil Extract of Inula helenium Root

In the Khatirchi district of Navoi region, the "Ubaydulla Ota" farm has been used to propagate Inula helenium L. in field conditions in two ways, the first is to sow seeds directly in the field and the second is to sow seedlings (planted in pots and raised) in field conditions. Before sowing, the seeds were soaked in a mixture of potassium permanganate and copper sulfate 0.002% solutions for 12 hours, and then the planting was carried out from this place.

1. A method for propagating Inula helenium L. from seeds to seedlings in field

conditions was developed and a raw material base was created for obtaining high-quality export products in the future.

2. For the production of a biologically active supplement based on the Inula helenium L. plant, a technical passport for the starting product Inula helenium L. plant was developed and approved by the Director of the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan, Academician K.Sh. Tojiboyev. To obtain the oil extract, 200 g of *Inula helenium* root is crushed and placed in a flask. Then, 500 ml of 96% ethanol is added, and extraction is carried out in a water bath at 50-60°C for 0.5 hours with the use of reverse cooling[7]. Afterward, 5 liters of sunflower oil are added, and the mixture is extracted at 50-70°C for another 0.5 hours, followed by standing for 1 hour. The extraction process continues at 95-100°C for 0.5 hours, during which ethanol is removed. The oil extract is then passed through a vacuum Nutsche filter under a pressure of 0.075 MPC and filtered, resulting in a light yellow oil extract of *Inula helenium* L.[8].

**Measuring Immunotropic Activity**

The immunotropic effects of the oil extract from *Inula helenium* root were assessed in vivo in mice using biological assays. The study analyzed the mass and nucleated cell (NC) count of immune organs, including the thymus, spleen, and axillary lymph nodes, as well as macrophage numbers in the peritoneal fluid. The experiment used mice weighing around 24 grams, with healthy animals receiving an oral dose of isotonic saline (0.9% NaCl), while experimental groups were administered a single dose of 200 mg/kg *Inula helenium* root oil extract in an oil solution[9]. The immunotropic activity of the extract was compared with the effects of Immunal tablets (containing 80 mg dry extract of *Echinacea purpurea* per tablet, produced by Sandoz, Slovenia) and Red May, an herbal product from Phytoleum LLC, Kazakhstan, which contains ingredients such as St. John's wort, nettle leaves, rose petals, licorice root, creeping thyme, lemon balm leaves, and sea buckthorn oil. After 48 hours, NCs were analyzed in immune organs from both healthy and experimental mice, and body weights were measured. To collect peritoneal fluid, 2 ml of 0.9% NaCl solution was injected intraperitoneally five minutes before the experiment. Pharmacotoxicological evaluations followed the specified model, with all procedures adhering to the European Convention for the protection of animals used in research, as per the European Directive 2010/63/EU, dated September 22, 2010, outlined in the Official Journal of the European Union L276/33-L276/79.

The animals were euthanized under ether anesthesia through decapitation, and 20 µL of peritoneal fluid was collected. The thymus, spleen, and axillary lymph nodes were removed, weighed, and ground in a 0.9% NaCl solution. Cell suspensions were then prepared by passing the ground tissue through a nylon sieve (20 ml solution per 100 mg of tissue). These suspensions were placed into test tubes with 0.4 ml of 3% acetic acid solution (diluted x20) using an automatic pipette (20 µL). Nucleated cells (NCs) were counted under a microscope at 10x40 magnification using a Goryaev chamber (model 851, TU 64-1-816-77), commonly used for blood cell counting. The NC count (T) was calculated with the formula $T = a \times v \times 4{,}000{,}000 / b$, where $a$ is the number of NCs in 100 large squares, $b$ is the NC count in small squares, $v$ is the dilution factor (x20), and 4,000,000 is the conversion factor for small square volume to 1 ml. The average NC number in immune organs was calculated with the formula $\text{NC}_{\text{avg}} = T \times V / n$, where $T$ is the NC count per ml of suspension, $V$ is the suspension volume in ml, and $n$ is the group size[10].

The effect of the oil extract of *Inula helenium L.* root on the cellularity of immune organs was determined by calculating the ratio of the average cellularity of immune organs in the experimental groups to that of the healthy group (Texp/Thlthy). This ratio was used to define the stimulation index (SI).

**Determination of Water-Soluble Vitamins in Inula helenium L. Root Using High-Performance Liquid Chromatography (HPLC)**

The water-soluble vitamins present in the root of *Inula helenium L.* were identified using the high-performance liquid chromatography (HPLC) method. To prepare the sample, 5-10 grams of the root were accurately weighed on an analytical balance and placed into a 300 ml flat-bottomed flask. Subsequently, 50 ml of 40% ethanol solution was added. The mixture was equipped with a magnetic stirrer and a reflux

condenser, and boiled with continuous stirring for 1 hour. It was then stirred for an additional 2 hours at room temperature. Afterward, the mixture was allowed to settle and filtered. The remaining solid was subjected to re-extraction twice by adding 25 ml of 40% ethanol each time. The filtrates were combined, transferred to a 100 ml volumetric flask, and diluted to the mark with 40% ethanol (5-10%). The resulting solution was centrifuged at 7000 rpm for 10 minutes. The supernatant was then taken for analysis[11].

Working solutions of water-soluble vitamins were prepared at a concentration of 1 mg/ml. For this purpose, 50.0 mg of each vitamin standard was accurately weighed on an analytical balance, dissolved in 50 ml of 40% ethanol in a volumetric flask, and diluted to the mark[12].

For HPLC analysis of water-soluble vitamins in *Inula helenium L.* root, an acetate buffer system and acetonitrile were used as eluents. The chromatography conditions were as follows:

• Chromatograph: Agilent-1200 (equipped with an autosampler)
• Column: Exlipse XDB C18 (reverse-phase), 5 µm, 4.6 x 250 mm
• Detector: Diode array detector (DAD), identified at 250 nm
• Flow rate: 0.8 ml/min
• Eluent composition: Acetate buffer: acetonitrile: 0-5 min 96:4, 6-8 min 90:10, 9-15 min 80:20, 15-17 min 96:4; thermostat temperature: 25°C
• Injection volume: 5 µl

**Table 1.** Effect of *Inula helenium L.* root oil extract on the number of macrophages in the peritoneal fluid of mice (M±m; n=6)

| Drug name, dose | Study time, hours | Macrophage count per $10^6$/ml | Promotion Index (PI) |
|---|---|---|---|
| A group of healthy animals | 48 | 110±9,49 | - |
| Immunal, 60 mg/kg | 48 | 114±10,14 | 1,03 |
| Red May | 48 | 90±7,87 | 0,81 |
| Oil extract of the root of *Inula helenium L.* plant | 48 | 176±2,76 p=0.000091 | 1,6 |

p<0,05 – compared to the results of a healthy group

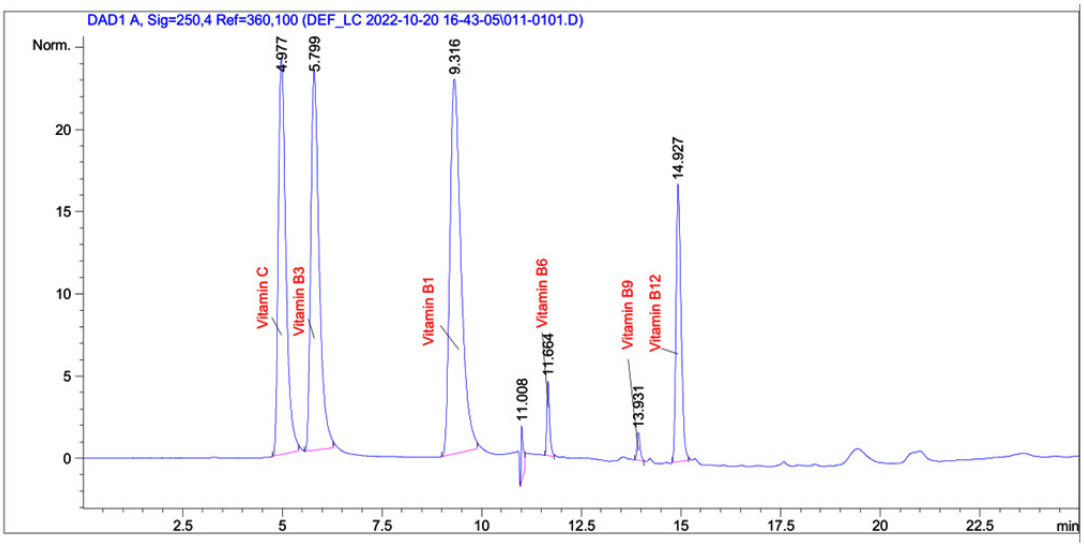


**Fig. 1.** Chromatography of the working standard solution to determine the amount of vitamins in the root of *Inula helenium L*

## RESULTS AND DISCUSSION

Initially, a working standard solution was prepared and analyzed on the chromatograph (Figure 1), followed by the introduction of the *Inula helenium L.* root extract (Figure 2). The two were compared, and the content of the vitamins was determined.

Water-soluble vitamins in the root of *Inula helenium L* plant were determined using high-performance liquid chromatography (HPLC) method. $B_1$=0.031896 mg., $B_2$=0.950895 mg., $B_6$= 0.621359 mg. in the root of Rubia tinctorum plant. , $B_9$= 1.868852 mg Vitamin C=0.249851 mg was found[11,12].

A comparative analysis of the effect of substances on phagocytes, which provide resistance to the animal organism, was carried out during the studies conducted to determine the immunotropic activity of the oil extract of the root of *Inula helenium L.* plant. Studies have shown that the number of macrophages in the peritoneal fluid of healthy mice was 110±9.49*$10^6$/ml. A single intragastric administration of the oil extract to animals at a dose of 200 mg/kg by catheter resulted in a 1.6-fold increase in the number of macrophages in the peritoneal fluid compared to animals in a healthy group[13,14]. The obtained results are presented in Table 1.

Also, the effect of the oil extract of the root of Inula helenium L. plant on the total number of cells of central (thymus) and peripheral (spleen, lymph nodes) immune organs was studied[15,16]. According to the data presented in Table 2, the effect of the oil extract of the root of Inula helenium L[17,18]. on the mass and number of T-cells in the thymus, which provides cellular immunity in the animal body, was 106 /thymus was 247.5±2.10.

The oil extract of the root of *Inula helenium L.* in a dose of 200 mg/kg was administered to animals once in the stomach, after 48 hours, the number of thymocytes in $10^6$/thymus increased by 1.3 times compared to healthy animals (402.67±0.46). This indicator shows the high stimulating activity of the oil extract of the root of *Inula helenium L.* plant. The third stage of our research was a comparative analysis of the effect of substances on the weight of the spleen and the number of CSF[13]. The conducted studies showed that the number of splenic cells in healthy animals was 209.17±14.52, and the weight of the spleen was 135.82±8.84 mg (Table 2). The oil extract of the root of the *Inula helenium L.* plant was administered to animals at a dose of 200 mg/kg

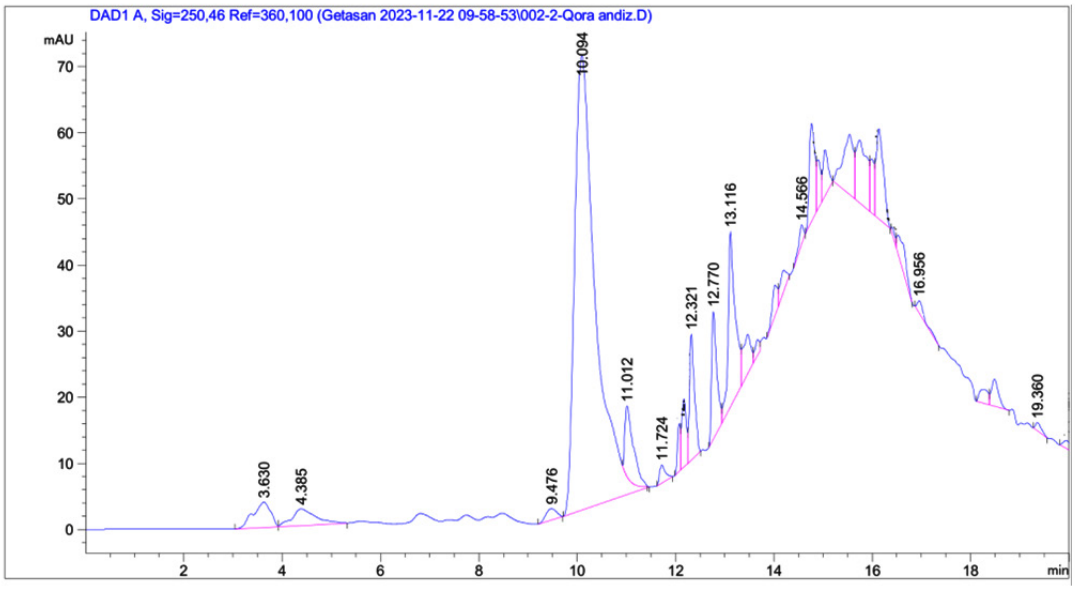


**Fig. 2.** Chromatography of a solution prepared from the root of *Inula helenium L* to determine the amount of vitamins in the root of *Inula helenium L*

**Table 2.** The effect of the oil extract of the root of *Inula helenium L.* and the comparative drugs on the mass of immune organs and the number of IHCs after oral administration (M±m; n=6)

| Research time - 48 hours | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Thymus | | | Black spleen | | | Lymph nodes | |
| mg | $10^9$/ Thymus | RI | mg | 10 $^9$/taloq | RI | mg | 10 $^9$/ lymph nodes | RI |
| **A group of healthy animals** | | | | | | | | |
| 27,77±0,65 | 296±5,84 | - | 135,82±8,84 | 209,17±14,52 | - | 12,48±0,59 | 215±21,31 | - |
| **Immunal, 60 mg/kg** | | | | | | | | |
| 58,90±3,91 | 475,83±4,15 | 1,6 | 213,85±29,3 | 314,17±25,16 | 1,5 | 18,85±1,59 | 107,5±4,18 | 0,5 |
| p=0.000026 | p=0.000000 | | p=0.031214 | p=0.005619 | | p=0.004512 | 0.000791 | |
| **Red May, 200 mg/ kg** | | | | | | | | |
| 48,05±6,08 | 223,17±16,40 | 0,7 | 294,12±21 | 207,5±17,82 | 0,99 | 10,07±1,12 | 310,83±17,4 | 1,4 |
| p=0.008988 | p=0.002364 | | p=0.000067 | | | | p=0.006903 | |
| **Oil extract of the root of *Inula helenium L.* plant, 200 mg/ kg** | | | | | | | | |
| 55,08±5,31 | 402,67±0,46 | 1,3 | 295,02±0,97 | 354,33±2,51 | 1,7 | 10,45±1,65 | 261±28,91 | 1,2 |
| p=0.000641 | p=0.000000 | | p=0.000000 | p=0.000004 | | | | |

p<0,05 – compared to the results of a healthy group

**Table 3.** The effect of the oil extract of the root of *Inula helenium L.* on the number of HCs in the bone marrow of animals (M±m; n=6)

| Drugs | Research time, hours | Bone marrow YXS $10^6$/ml $10^6$ | Promotion Index (PI) |
|---|---|---|---|
| Immunal, 60 mg/kg | 48 | 2728,33±169,24 p=0.005098 | 1,3 |
| Red may | 48 | 2761,83±48,33 p=0.000070 | 1,3 |
| Oil extract of the root of *Inula helenium L.* plant | 48 | 3458,33±68,29 p=0.000001 | 1,7 |

Appendix: bone marrow of healthy animals - 2010±97.57*$10^6$/ml (48 hours)

once, after 48 hours, the number of splenocytes increased by 1.7 times compared to the group of healthy animals[14]. At the same time, the weight of the spleen also has a tendency to increase compared to the spleens of animals in the healthy group and the group of animals that used the comparative drug Immunal[15]. The fourth stage of our research is characterized by a comparative analysis of the effect of the oil extract of the studied *Inula helenium L*[16]. plant on the weight of lymph nodes and the number of CSFs[17]. Research showed that in intact animals, the number of lymph node YXS was 215±21.31, and the weight of the lymph node was 12.48±0.59 mg (Table 3).

In the fifth stage of immunological studies, the effect of the oil extract of *Inula helenium L*[19,20]. and the comparative drugs on the number of IH in the bone marrow of mice was studied (Table 3)[21]. The oil extract of the root of *Inula helenium L.* significantly stimulated the increase of YaH in the bone marrow of mice (RI=1.7), where the PI was higher than that of the comparative drugs[22].

## CONCLUSION

1. Using high-performance liquid chromatography (HPLC) to analyze the water-soluble vitamin content in the root of the *Inula helenium* plant, the following concentrations were identified: Vitamin B1 at 0.0319 mg, B2 at 0.9509 mg, B6 at 0.6214 mg, B9 at 1.8689 mg, and Vitamin C at 0.2499 mg per gram of root.

2. An oil extract was obtained from the root of *Inula helenium L.* based on sunflower oil - Oleum Heuanth1. This oil is a light red oily liquid with a characteristic smell, miscible in ethanol, glycerin, some organic solvents, and it has been found that it forms a white cloudy color in water.

3. *Inula helenium* root extract in sunflower oil was found to have a stimulating effect on the non-specific resistance (phagocytes) and all lymphoid organs of the animal organism at a dose of 200 mg/kg once orally, i.e. after 48 hours significantly increased the weight of the spleen (PI=1.7), the thymus, and the number of HCV. It has a stimulating effect on the central organ of immunity, the thymus (PI=1.3) and the peripheral organ - the lymph nodes (PI=1.2). can be recommended for use.

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The author(s) do not have any conflict of interest.

**Data Availability Statement**

This statement does not apply to this article.

**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials

**Author Contributions**

Ozoda Gaibullayeva – Conceptualization, methodology, investigation, and writing the original draft. Akmal Islomov – Methodology, data analysis, and writing review and editing. Dilnoza Abdugafurova – Investigation, data curation, and visualization. Boynazar Elmurodov – Methodology and resources. Baxodir Mirsalixov – Data analysis and interpretation. Lazizbek Mahmudov – Investigation and manuscript preparation. Izzatullo Adullaev – Writing review and editing, and supervision. Sitora Sadullayeva and Ko'zijon Baratov – Resources and data management. Sirojiddin Omonturdiev – Supervision of experiments and project administration.

## REFERENCES


1. Azimova, L. B., Filatova, A. V., Vypova, N. L., & Turaev, A. S. mmunotropic activity and acute toxicity of melanin isolated from horse chestnut (Aesculus hippocastanum L.) seed coats. *Pharmaceutical Chemistry Journal,* 2024, *58*(15).
2. Khaytmetova, S. B., Turaev, A. S., Khalilova, G. A., & Muhitdinov, B. I. Isolation, physico-chemical characteristics, and acute toxicity evaluation of water-soluble polysaccharide from basidial raw material *Ganoderma lucidum*. *Russian Journal of Bioorganic Chemistry, 2024, 50*(1), 95-105.
3. Weichbrod, R. H., Thompson, G. A. H., & Norton, J. N. Management of animal care and use programs in research, education, and testing. CRC Press. 2017, 15(2). https://doi.org/10.1201/9781315152189
4. Pan, S.-Y., Zhou, S.-F., Gao, S.-H. New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. *Evidence-Based Complementary and Alternative Medicine, 2013*, 627375.
5. Abdullaev, A. A., Inamjanov, D. R., Abduazimova, D. Sh., Omonturdiyev, S. Z., Gayibov, U. G., Gayibova, S. N., & Aripov, T. F. Sílybum mariánum's impact on physiological alterations and oxidative stress in diabetic rats. *Biomedical and Pharmacology Journal*, 2024, 17(3), 1291-1300.
6. Zoirovich, O. S., Ugli, A. I. Z., Raxmatillayevich, I. D., Umarjonovich, M. L., Ravshanovna, Z. M., Narimanovna, G. S., Gapparjanovich, G. U., & Fatikhovich, A. T. The effect of Ájuga turkestánica on the rat aortic smooth muscle ion channels. *Biomedical and Pharmacology Journal*, 2024, 17(2).
7. Sigma-Aldrich. Chemical reagents for research and analysis. Available at: www.sigmaaldrich.com.
8. Agilent Technologies. Application note: HPLC analysis of vitamins. Available at: www.agilent.com.
9. Harborne, J. B. *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer Science & Business Media.
10. Paviani, L. C., & Dariva, C. Extraction of essential oils from medicinal plants using supercritical CO2: Experiments and modeling. *Journal of Supercritical Fluids, 2010, 54*(1), 11-17.
11. Zhao, C., & Li, Y. Comparative study on immunomodulatory activities of *Inula helenium* extracts. *International Immunopharmacology, 2021, 90*, Article 107188.
12. Lin, C.-C., & Huang, P.-C. Pharmacological and immunological effects of herbal extracts: A study on traditional Chinese medicine. *Chinese Medicine,2020, 15*(1), 42.
13. Gómez, M. R., & Silva, M. F. High-performance liquid chromatography (HPLC): Methodology and applications in the analysis of plant secondary metabolites. *Phytochemical Analysis, 2007, 18*(2), 111-118.
14. Ermakova, A., & Shubina, E. E. Quantitative determination of vitamins in herbal preparations by HPLC. *Journal of Analytical Chemistry, 2022, 77*(5), 451-456.
15. Mehta, P. M., & Patel, A. A comparative study of plant extracts on the enhancement of immune system functionality. *Journal of Herbal Medicine, 2020, 23,* Article 100365.
16. Vasileva, A. G., & Kutyrev, G. I. The influence of plant-based oils on the immune response in rodents: A comprehensive study. *Journal of Biological Sciences, 2021, 17*(3), 264-271.
17. Saydullayevna, I. A., Gulyamovna, A. D., Hushvaqovich, I. A., Umarjonovich, M. L., Ugli, B. K. R., & Kizi, A. A. Q. The study of the biologically active effect of *Rubia tinctorum* L. plant on rats with experimental kidney stone disease and issues of introduction. *Biomedical and Pharmacology Journal, 2024, 17*(3).

19. Nakagawa, M., Ohgami, M., Ando, N., Wakabayashi, G., & Kitajima, M. Effects of steroids on the lung accumulation of neutrophil and monocyte in rabbits with endotoxemia. *Chest*.
20. Huang, H., Li, P., Ye, X., Zhang, F., Lin, Q., Wu, K., & Chen, W. Isoalantolactone increases the sensitivity of prostate cancer cells to cisplatin treatment by inducing oxidative stress. *Frontiers in Cell and Developmental Biology*.
21. Meng, Z., & Liu, Y. Bioactive compounds and pharmacological effects of *Inula helenium*: A review. *Journal of Ethnopharmacology, 2020, 255*, Article 112675.
22. McKenna, D. J., Jones, K., Hughes, K., & Tyler, V. M. *The desk reference for major herbal supplements; Botanical medicines 2019(2)*.