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Isolation and Study of Dry Extract from *Echinacea Purpurea*

Z.A. Zuparova^α & G.M. Ismoilova^σ

Abstract- Medicines based on echinacea can be attributed to the group of herbal remedies that increase the activity of nonspecific factors of the body's defence and the immune system. The stimulation of bone marrow haematopoiesis with echinacea preparations increases the number of leukocytes and spleen RES cells. Echinacea preparations have a mild, multivalent effect and practically no side effects. A dry extract was obtained from the herb *Echinacea purpurea* by polyextraction.

The conditions for extraction and the extractants were selected, and the kinetics of extraction, isolation and purification of the extract from the herb *Echinacea purpurea* were studied.

Based on the studies carried out, the extract was found to contain flavonoids, hydroxycinnamic acids, tannins, amino acids, and polysaccharides.

The polysaccharide hydrolysate contained monosaccharides such as uronic acids, galactose, trace amounts of glucose, arabinose, xylose, and sucrose from ketosaccharides. The main monosaccharides making up the polysaccharide were uronic acids and arabinose.

Keywords: *echinacea purpurea*, dry extract, extractants, extraction kinetics, flavonoids, hydroxycinnamic acids, polysaccharides, monosaccharides.

I. INTRODUCTION

Currently, there is a wide range of secondary immunodeficiencies associated with urbanization, chemization, and increased stress load, leading to disruptions in the functioning of the immune system. Such conditions require immunocorrection. Substances that stimulate the body's nonspecific defence must be effective, available, and harmless. In this regard, a special place is occupied by research related to the introduction of herbal medicines into medical practice, the study of their chemical composition, and the optimization of technologies for obtaining such medicines. Herbal remedies have been used in medicine as long as the concept of treating diseases has existed. For thousands of years, mankind has used herbal medicines for therapeutic purposes. Vegetable raw materials are environmentally friendly, and their use is based on the close relationship of the human body and natural components. The search for new types of

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raw materials and the study of previously used plants as potential sources for obtaining drugs with immunomodulatory action is relevant [1,2]. Medicines based on echinacea belong to the group of herbal remedies that increase the activity of nonspecific factors of the body's defence and the immune system. The stimulation of bone marrow haematopoiesis with echinacea preparations increases the number of leukocytes and spleen RES cells. Echinacea preparations have a mild, multivalent effect and practically no side effects [3,4].

Echinacea preparations are used in medical practice as immunomodulatory agents. The biologically active substances they contain activate the protective cells of the immune system - phagocytes. Preparations of this plant, in addition to immunomodulatory properties, are used in the treatment of tumours. Juice from fresh inflorescences effectively promotes blood clotting and wound healing.

In folk medicine, echinacea is used for colds, flu, blood poisoning, diseases of the bladder, urticaria, burns, herpes, heavy metal poisoning, liver diseases and diabetes. Echinacea-based preparations have a depressing effect on streptococci, *Escherichia coli* and the influenza virus.

II. MATERIALS AND METHODS

To obtain a dry extract by polyextraction, dried *Echinacea purpurea* was used as a starting material. Qualitative assessment of flavonoids was carried out by TLC and chemical-analytical reactions. TLC was carried out on "Mesk" chromatographic plates with 60 F 254silica gel on an aluminium substrate (10X15 cm) in the solvent system n-butanol - acetic acid - water (4: 2: 1), with standard samples of rutin, luteolin, and quercetin for comparison. Zones of adsorption were detected under UV light at a wavelength of 254 nm. After chromatography, the chromatographic plate was dried in a drying oven at a temperature of 100-105 °C (Table 1).

Additionally, for the qualitative detection of flavonoids, reactions with caustic soda were carried out. The qualitative detection of oxycinnamic acids was carried out by the paper chromatography method in a chromatographic chamber with 2% acetic acid as a mobile phase. Tannins were detected with ammonium iron alum. Qualitative detection of amino acids was carried out using the ninhydrin reaction. Qualitative

detection of alkaloids was carried out using silicotungstic acid.

III. RESULTS

Selection of extractant. The extraction process of the dark red echinacea surface was studied to select a moderate extractant. Purified water and 40%, 70%, and 96% ethyl alcohol were used as extractants. Then, 0.5 kg of raw material was placed in an extractor with a

volume of 5 l, and the extractant was poured in until a glassy surface was formed. The extraction was carried out at room temperature. Every 8 hours, the extract in the extractor was poured out, and fresh solvent was poured in until a glassy surface was formed on the surface of the raw material. This process was repeated 4 times. The extracts from each extractor were combined and determined based on the content of chlorogenic acid, polysaccharides and extractives (see Figure 1).

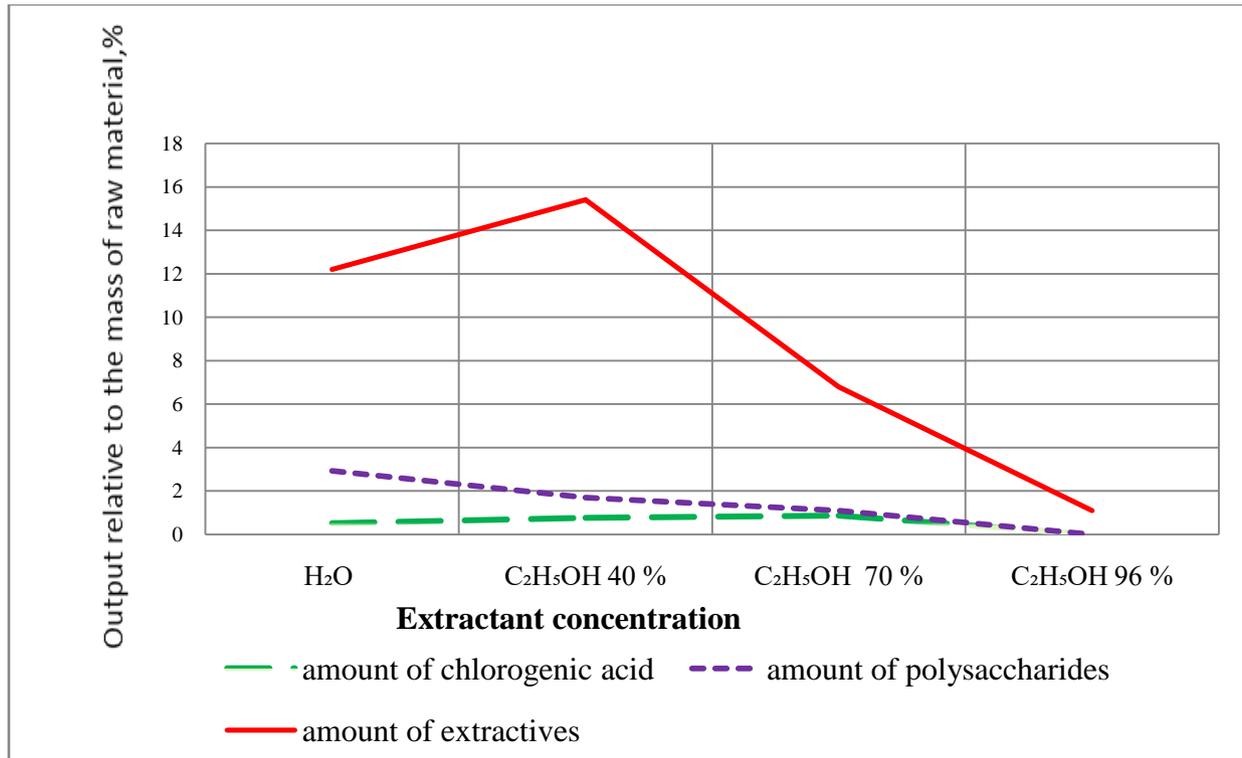


Figure 1: Influence of extractant type on the release of biologically active substances

The curves in the figure show that the amount of chlorogenic acid is highest in the extract obtained in 70% ethyl alcohol, the amount of polysaccharides was highest in the aqueous separation, and the amount of extractives was highest in the separation in 40% ethyl alcohol. For this reason, the polyextraction method was selected as a moderate method to obtain a dry extract with an immunomodulatory effect from the dark red echinacea plant.

Selection of raw material fineness level. The raw material of dark red echinacea was crushed and passed through sieves of different diameters. Then, 0.5 kg of raw materials of different fineness levels was taken from each batch and placed in 5 extractors with a capacity of 5 l. Raw materials smaller than 2 mm were placed in the first extractor, 2-5 mm in the second extractor, 5-8 mm in the third extractor, 8-11 in the fourth extractor, and more than 14 mm in the fifth extractor. Extraction was performed 3 times, first with 70% ethyl alcohol, then with 40% ethyl alcohol, and finally with water. Every 8 hours, the separation was removed and combined. The

combined extracts were tested for the amount of flavonoids and extractives (see Figure 2).

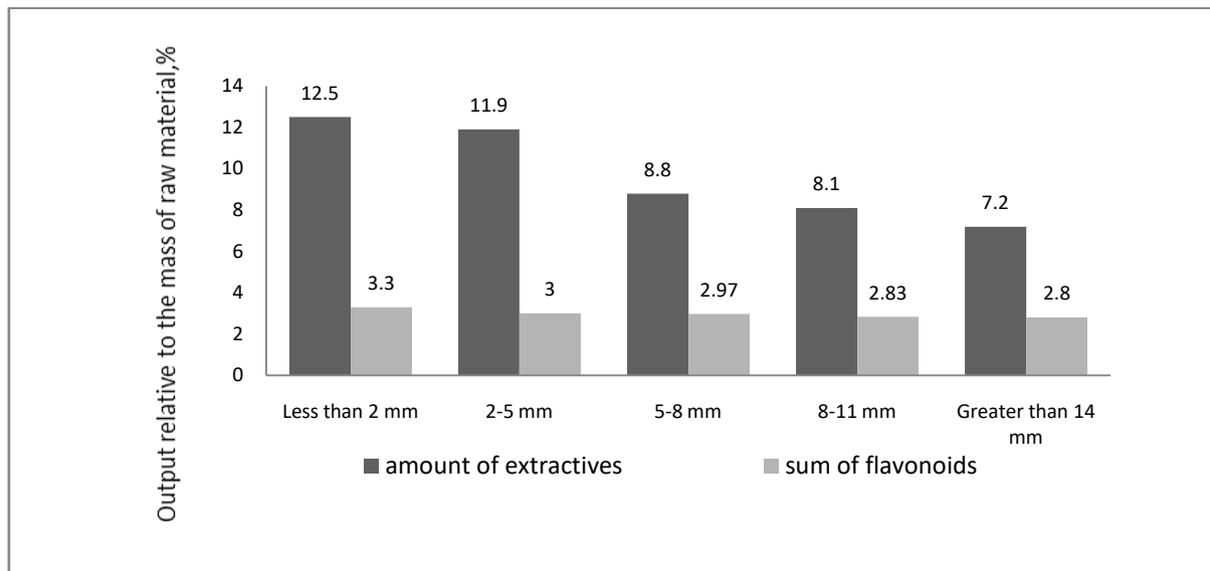


Figure 2: The effect of the degree of fineness of the raw material on the release of flavonoid aggregates

The rate of separation of flavonoids from the 2 mm raw material was high, but the resulting extract was turbid and difficult to filter. The extraction rate from the raw material was slow. The yield of flavonoids from crushed raw materials of 5-8 mm and 8-11 mm was lower than that of crushed raw material of 2-5 mm. To isolate flavonoids, a dark red echinacea raw material of 2-5 mm in size was selected as suitable.

Temperature selection. The temperature factor plays an important role in the separation of biologically active substances. As the temperature increases, the extraction rate also increases due to the increase in the diffusion rate.

To select the most appropriate temperature, 2-5 mm crushed raw material was placed in 4 extractors with a volume of 0.5 kg to 5 l and treated with 70% ethyl alcohol until a glassy surface was formed. Extraction in the first extractor was carried out at room temperature of 20-30 °C, in the second extractor at 30-40 °C, in the third extractor at 40-50 °C and in the fourth extractor at 50-60 °C. Extraction was not carried out at temperatures above 60 °C, as the efficiency of separation of biologically active substances was almost unchanged (see Figure 3).

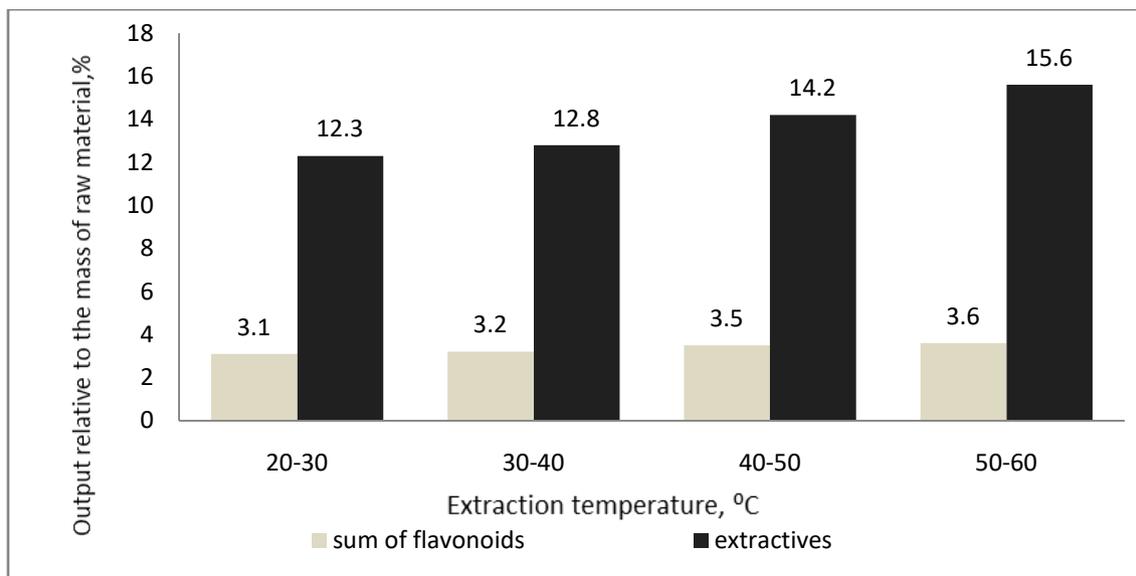


Figure 3: The effect of temperature on the level of flavonoid release

According to the data in the picture, the release of extractives at the temperature of 50-60 °C was the highest, but the total flavonoids was almost from the same as that obtained at 20-40 °C. Accordingly, the

separation of flavonoid aggregates from dark red echinacea raw material at room temperature was found to be appropriate.

Purification of the extract. Extraction in a liquid-liquid system is the most widely used extract purification method. Therefore, to purify the concentrated alcoholic extract of dark red echinacea, nonaqueous organic solvents such as hexane, acetone, chloroform, and extraction gasoline were used. The experiment was carried out as follows: 0.5 kg of crushed raw material with a size of 2-5 mm was placed in an extractor with a volume of 5 l. It was first extracted with 70% ethyl alcohol, then with 40% ethyl alcohol, and then with purified water for 5 hours. The resulting extracts were combined and divided into 4 equal parts. Each part of the extract was concentrated at a temperature of 60-700 °C and a vacuum of -0.6...- 0.4 kgf/cm³ to 1.2 l. Water was added in a 1:1 ratio. The first aqueous solution was treated 3 times with 0.5 l of hexane, the second with acetone, the third with chloroform, and the fourth with extraction gasoline. The separations in each reactor were analysed. The results of the analysis revealed a high degree of clarity of the extract treated with chloroform and a low content of lipophilic substances and chlorophyll.

Obtaining dry extract of dark red echinacea by polyextraction.

Five kilograms of 2-5 mm air-dried, weighed dark red echinacea was placed in a KD-2KY extractor and covered with 70% ethyl alcohol until a glassy surface was formed. The extractor was hermetically sealed and extracted by suffocation for 6 hours. Extraction was carried out at room temperature. After the

allotted time, 15 l of the first separation was poured off. The extraction was repeated by adding 40% ethyl alcohol to the extractor until a glassy layer was formed on the surface of the raw material. After 6 h, 15 l of the separation was poured off. For the third extraction, purified water was poured over the raw material. Extraction took 6 hours. *The third separation was 15 l. The first, second, and third separations were combined and transferred to the collector through a multilayer fabric filter.* The filtered separation was evaporated from 20-25 l in a rotor vacuum evaporator at 70-80 °C and -0.8-0.4 kgf/cm². A total of 11.2 l of concentrated alcohol extract was poured from the vacuum evaporator into the reactor and diluted with 10 l of water. The resulting aqueous extract was treated 3 times with 1.0 chloroform. The purified separation in chloroform was evaporated in a rotary vacuum evaporator at 50-60 °C and -0.6-0.8 kgf/cm². The purified extract was then dried in a spray dryer.

IV. DISCUSSION

A greenish brown powder with a characteristic odour was isolated. A phytochemical study of the isolated dry extract from the herb *Echinacea purpurea* was carried out to create an effective drug or standardized extract with pharmacological effects, including immunomodulatory effects.

The data obtained are shown in the Table 1.

Table 1: The qualitative composition of some biologically active substances in the dry extract of *Echinacea purpurea*

Substances	Analysis methods and conditions	Analytical effect of reaction
1	2	3
Flavonoids	Reaction: with caustic soda	Yellow staining
Hydroxycinnamic acids	Paper chromatography method, mobile phase 2% acetic acid solution	Blue fluorescence of spots under UV light
Tannins	Reaction with ammonium iron alum	Dark green colouration
Amino acids	Reaction with 0.1% ninhydrin	Red and purple spots

The studies carried out on the extract revealed the presence of flavonoids, hydroxycinnamic acids, tannins, and amino acids [5,6].

The immunomodulatory effect of *Echinacea purpurea* preparations is due to the sum of biologically active substances, but the main immunomodulatory effect is due to polysaccharides [7,8,9].

The following fractions were isolated:

- Fraction of polysaccharides extracted with water at room temperature (VRPS-X) (ratio 1:20, precipitant 95% ethyl alcohol 1:3);
- Fraction of polysaccharides extracted with hot water (VRPS-G) (temperature 80 °C, ratio 1:20, precipitant 95% ethyl alcohol 1:3);
- Fraction of pectin substances (PV) (equal volumes of 0.5% solutions of oxalic acid and ammonium

oxalate, ratio 1:20, temperature 70 °C, precipitant ethyl alcohol 1:5);

- fraction of hemicelluloses (HC) (0.5% potassium hydroxide, temperature 20 °C, ratio 1:20, precipitant ethyl alcohol 1:4).

The isolated fractions were subjected to acid hydrolysis (water-soluble polysaccharides were hydrolysed with 1 N H₂SO₄ for eight hours at 100 °C, pectin substances and hemicellulose-2n H₂SO₄ for 20 hours at 100 °C), neutralized with barium carbonate, demineralized using the KU-2 cation exchanger in H⁺ form. Then, monosaccharides were identified by paper chromatography and comparison with standard samples. Paper chromatography was performed on Filtrak-FN 18 paper in a butanol-1-pyridine-water (6: 4: 3) solvent system (system 1). Aniline phthalate acid

(developer 1) and a 5% urea solution (developer 2) were used to identify spots. Chromatograms were developed at 105-110 °C.

Water-soluble polysaccharides - the monosaccharide composition of the polysaccharide represented by uronic acids and neutral monosaccharides (system 1, developer 1).

Pectin substances- monosaccharide composition represented mainly by uronic acids, galactose, and arabinose (system 1, developer 1).

Hemicellulose structural components including uronic acids and neutral monosaccharides (system 1, developer 1), mainly galactose; arabinose and xylose are less pronounced in paper chromatography.

The polysaccharide hydrolysate contained monosaccharides such as uronic acids, galactose, trace amounts of glucose, arabinose, and xylose. The main monosaccharides were uronic acids and arabinose.

When determining water-soluble polysaccharides, 0.5 ml of purified water was added to 20 ml of liquid extract, and hydrolysis of the polysaccharide was carried out for 12 hours at 100 °C with 1 N sulfuric acid. The hydrolysate was neutralized with BaCO₃, filtered off and deionized with a KU-2 (H⁺) cation exchanger, evaporated and chromatographed in a butanol-pyridine-water solvent system at a ratio of 6:4:3 for 18 hours. The chromatogram was dried and treated with acidic aniline phthalate, revealing the presence of the following monosaccharides: uronic acids, galactose, trace amounts of glucose, arabinose, xylose, and from ketosaccharides, sucrose. The main monosaccharides were uronic acids and arabinose.

V. CONCLUSION

The conditions for extraction and the extractants were selected, and the kinetics of the extraction, isolation and purification of the extract from the herb *Echinacea purpurea* were studied.

Based on the studies carried out, the extract was found to contain flavonoids, hydroxycinnamic acids, tannins, amino acids, and polysaccharides.

The polysaccharide hydrolysate was found to contain monosaccharides such as uronic acids, galactose, trace amounts of glucose, arabinose, and xylose, as well as sucrose from ketosaccharides. The main monosaccharides that make up the polysaccharides are uronic acids and arabinose.

REFERENCES RÉFÉRENCES REFERENCIAS

1. Bizunok N.A. Pharmacological properties of echinacea // Recipe. 2008. No. 5. p. 42-49.
2. Zuparova Z.A., Olimov N.K., Ismoilova G.M., Khasanova B.J. Determination of high quality of *Echinaceae purpurea* herb grown in Uzbekistan and the respect of creating immunomodulatori

3. Barnes, J., Anderson, L.A., Gibbons, S. and Phillipson, J. D. (2005) *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): A Review of Their Chemistry, Pharmacology and Clinical Properties. *Journal of Pharmacy and Pharmacology*, 57, 929-954. <http://dx.doi.org/10.1211/0022357056127>.
4. Cardinale, M., Viola, M., Miceli, E. *et al.* The cypsela (achene) of *Echinacea purpurea* as a diffusion unit of a community of microorganisms. *Appl Microbiol Biotechnol* 105, 2951–2965 (2021). <https://doi.org/10.1007/s00253-021-11212-2>.
5. Rezaei, E., Abedi, M. Efficient Ultrasound-Assisted Extraction of Cichoric Acid from *Echinacea purpurea* Root. *Pharm Chem J* 51, 471–475 (2017). <https://doi.org/10.1007/s11094-017-1635-y>.
6. Brykalov A.V., Golovkina E.M., Belik E.V., Bostanova F.A. Study of physiologically active compounds in a preparation from *Echinacea purpurea* // *Chemistry of plant raw materials*. 2008. No. 3 p. 89-91.
7. Wagner H., Stuppner H., Schafer W., Zenk M.A., Immunologically active polysaccharides of *Echinacea purpurea* cell cultures, *Phytochemistry*, 1988, 27, 119–126.
8. Glavač, N.K., Košir, I.J., Rode, J. *et al.* Optimization and use of a spectrophotometric method for determining polysaccharides in *Echinacea purpurea*. *cent.eur.j.biol.* 7, 126–131 (2012). <https://doi.org/10.2478/s11535-011-0091-z>.
9. Classen B., Witthohn K., Blaschek W., Characterization of an arabinogalactan-protein isolated from pressed juice of *Echinacea purpurea* by precipitation with the beta-glucosyl Yariv reagent, *Carbohydr. Res.*, 2000, 327, 497–504.