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Research Paper

Chemical Composition and Organoleptic Properties of a Tincture made from Echinacea and Leuzea

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Abstract

Medicinal plants are the major sources of vitamins, minerals, salts, macro-/micro- nutrients and bioactive compounds that have beneficial effects on human health. In the context of current pandemic, this becomes even more important. The purpose of this work is to obtain a bioactive food supplement with immunomodulatory effect. The technology of obtaining a tincture from a 1:1 composition of Echinacea and Leuzea is developed using mathematical simulation, its chemical composition and organoleptic properties are characterized. Method of extraction of bioactive compounds from plants and its analysis by gravimetric, chromatographic and foaming methods are elucidated. The tincture contains 7.9% flavonoids, 7.5% tannins and 8.3% saponins. Among the essential amino acids present in the tincture, lysine, phenylalanine, leucine and isoleucine predominates. The heavy metal concentration is within the maximum allowable limit. This herbal formulation may have immense application potential in the area of nutritional supplements, immunity booster, functional food and disease prevention

Keywords: Medicinal plant, bioactive food supplement, flavonoids, tannins, saponins, tincture, herbal formulation.

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1. Introduction

In the East Kazakhstan region, one of the risk factors for disease, namely cancer, is the contamination of the environment, food raw materials and foodstuffs with radioactive elements and heavy metals, including the consequences of the Semipalatinsk nuclear test site [1, 2]. Subsequently, attention must be paid to the production of preventive food products that are produced using medicinal plants with immunomodulatory effect. To reduce the side effects of traditional methods of treatment (radiation and chemotherapy) of cancer, it is recommended to use various biologically active additives derived from medicinal plants with immunomodulatory properties [3, 4]. In the context of current pandemic, herbal supplements have become even more important to boost immunity and health, the whole world is turning towards it. In the production of food products, medicinal plants are used in the form of extracts, infusions, broths and syrups [5, 6].

For getting a tincture from medicinal plants, a formulation using two species of medicinal plants growing in the East Kazakhstan region was made. The formulation includes: Echinacea (*Echinacea purpurea*) and Leuzea (*Leuzea carthamoides*). The

combination of two medicinal plants is justified by the fact that all plants, as shown by studies of several scientists [7-9], contain biologically active substances that increase the immune status of human exposure to adverse factors. These substances include flavonoids, alkaloids, tannins, vitamin C and others.

The use of Echinacea purple (*Echinacea purpurea*) is associated with a high content of biologically active substances and a wide range of its preparations. At present, Echinacea purple is used to produce more than 30% of all medicinal products. Many studies have been conducted to assess the impact of the biological and immune activity of Echinacea purple on animals and humans [10, 11].

Preparations based on Echinacea purple (*Echinacea purpurea*) and Echinacea pale (*Echinacea pallid*) are actively used to prevent immune system disorders, as they have a positive effect on the body's immunity to infectious agents, reduce allergic diseases, and promote rapid healing of burns, wounds, and ulcers [12, 13].

Preparations based on various alcoholic and alcohol-water tinctures of Echinacea purpurea are used to restore and increase the immune response of the body. These preparations stimulate cellular and humoral immune reactions by activating phagocytosis, increasing the bactericidal activity and cytotoxicity of macrophages, and strengthening antibody synthesis [14].

Maral root (*Leuzea carthamoides*) normalizes the synthesis and accumulation of energy sources in the body - adenosine triphosphoric acid, glycogen and creatine phosphate [15]. The maral root is usually used as a finely ground powder. It is characterized by bitter taste, brackish resinous smell, spider-powdered fibrous inclusions, and high level of phytoexdysteroids. The concentration of ecdysteroids is higher than in other plants up to 100 times. Small doses of maral root have stimulating, anti-inflammatory and inhibitory effects [16, 17].

The aim of the work is to study the physical and chemical parameters, amino acid composition and organoleptic properties of the tincture from the composition of Echinacea and leuzea.

2. Experimental

2.1. Materials and methods

Lab experiments were carried out in the departments of Technology of food and processing industries, Biotechnology and standardization, Scientific center of radioecological research of the Shakarim State University of Semey, Kazakhstan, and Research institute of biotechnology of Kemerovo State University, Russia.

The leuzea and echinacea plants were sourced from the East Kazakhstan region, Katon-Karagai district and Shemonaikha district, respectively.

For preparation of a tincture an aqueous solution of ethyl alcohol of different concentration was used. It is recommended to use 35% aqueous solution of ethyl alcohol to make a medicinal plant tincture. To prepare the tincture using pharmacopoeia medicinal plants that are not poisonous plants: one part of the plant mass is mixed with 5 parts of the extractant. The infusion temperature of the infusion should be within 20-25 °C. Purple Echinacea grass and leuzea root were used as plant materials. To prepare the tincture, each plant species was dried to a constant weight at room temperature without sunlight. They were grinded to small particles 3-5 mm in size. Then mixed in a ratio of 100 g Echinacea and 100 g Leuzea and added to a water solution of 35% ethyl alcohol. The grinded grass was loaded into a mesh basket, which was placed in a percolator. Percolator is equipped with a jacket, for the possibility of maintaining the temperature of maceration in the range from 20 to 30 ° C.

A basket of grass was loaded into the percolator and 35% ethyl alcohol extractant was added in the ratio of herb : extractant 1:5. Then this mixture was infused in a closed apparatus at a temperature of 20 to 30 ° C for 12-24 hours. Then the content of the percolator was stirred for at least 1 hour, increasing the temperature up to 40-50°C. Then again infused for 12-24 hours and drained part of the extract, equal to the amount of herbs to be loaded. After draining the extract, a fresh extraction solvent is added to the treated herbs in the ratio of herb : extractant 1:1. Infusion with periodic mixing is carried out for 12 hours and all of the extract was drained into an intermediate container.

All received extracts are combined in the tank. To purify the resulting mixture of ballast substances, the process of sedimentation is carried out at a temperature of 5-8 ° C for 5 days, then the process of filtration of alcoholic infusion through a cotton-grass filter is performed (Figure 1). The resulting tincture is stored in dark glassware**.**

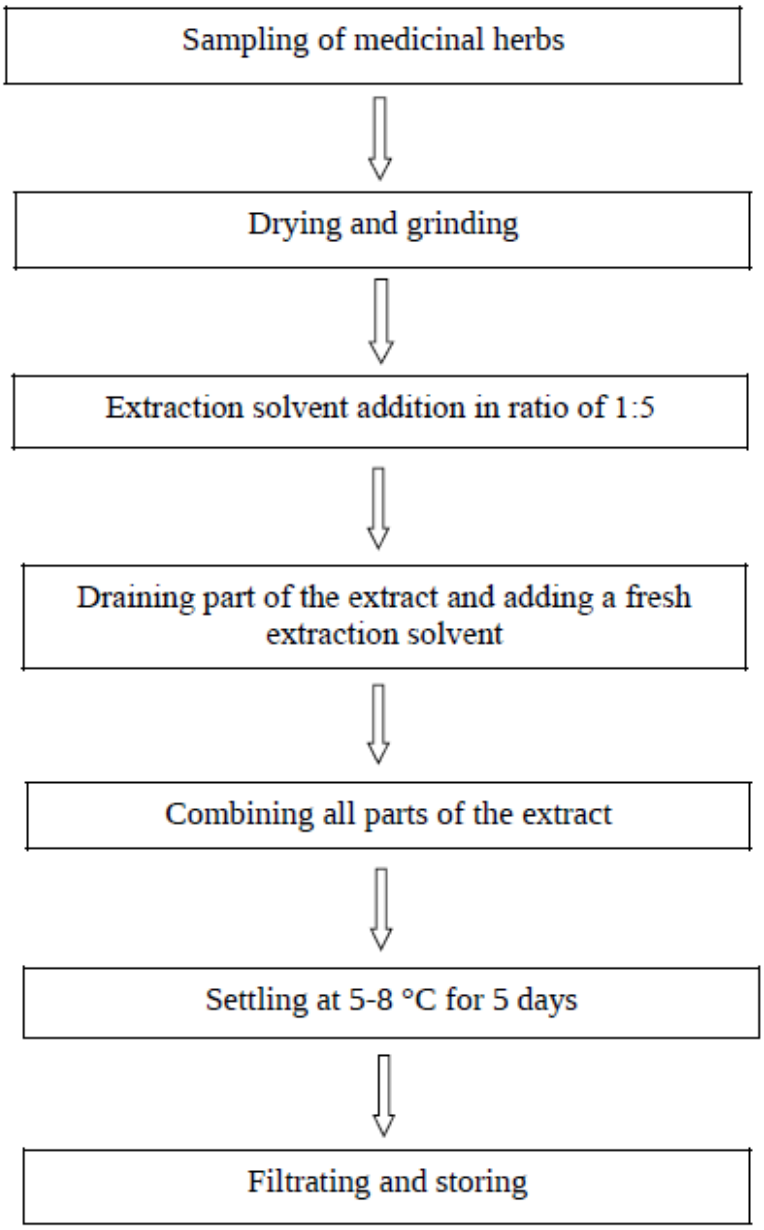

Figure 1. Flow diagram for obtaining the tincture.

2.2. Analytical Methods

Methods for the determination of chemical composition of medicinal plants were analyzed according to this reference [18].

2.3. Qualitative determination of saponins using a foam reaction

For conducting the reaction, water extraction from medicinal plants is divided into two parts: the first is acidified to pH=1, the second is alkalized to pH=13. Both solutions should be shaken in tubes. The formation of foam columns is observed. If in both tubes foam columns are formed approximately equal in size and resistance or in the tube with acidic medium, the tested sample contains triterpene saponins, if the foam column is larger at alkaline pH, then it is steroid saponins.

2.4. Determination of tannins, flavonoids and amino acids

For conducting the reaction, water extraction from medicinal plants is divided into two parts. One part is steamed, dried and weighed. The second part is treated with skin (hide) powder, filtered. The filtrate is evaporated to dryness, then it is dried and weighed. By the difference of dry residue in the control and in the experiment the content of tannins is determined.

Flavonoid quantification was determined by chromatographic method [19]. Amino acid composition was determined using GCMS-QP 2010 Ultra chromatomass spectrometer, M-04-38-2009 test method (FR.1.31.2010.07015) [20].

3. Results and Discussion

The aim was to obtain the optimal ratio of leuzea and echinecea in the herbal formulation. The results of the study are presented in Table 1. As can be seen from Table 1, tannins are found in both of the plants studied, and the largest amount is found in the roots of the leuzea. Tannins have a wide range of pharmacological action, including antioxidant properties, increasing the resistance of the body to adverse environmental factors. The greatest quantity of flavonoids is found in echinacea. Flavonoids also have a wide range of pharmacological action, including antioxidant and immunostimulating effects [21-23].

Table 1. Chemical composition of medicinal herbs.

Name of medicinal herb	Indicator	% wt	Daily rate for human, mg
Leuzea	Flavonoids	3.1	25
	Tannins	5.5	10
	Saponins	5.6	10
Echinacea	Flavonoids	5.5	25
	Tannins	3.1	10
	Saponins	4.1	10

Mathematical simulation using MS Excel was performed to determine the ratio of leuzea to echinacea. We enter data from Table 1 into MS Excel using a function:

$$F(x)= 0.5\ x_1+0.5.x_2 \rightarrow \text{const}$$

Using the "Solution Finder" function of the solution we set limits. On the basis of the obtained mathematical model we obtain the ratio 1:1. That is, it is established that to create a composition with a balanced composition of biologically active substances, we need a ratio of leuzea and echinacea 1:1 respectively.

On the basis of the above, in order to create a composition with a balanced composition of biologically active substances, a study with a different ratio of medicinal plants was conducted. As a result, the ratio of leuzea and Echinacea was chosen to be 1:1 respectively.

Organoleptic and physico-chemical indicators were analyzed in the obtained tincture. The results of the study are presented in Table 2. As can be seen from Table 2 on organoleptic physico-chemical indicators, the tincture corresponds to the requirements of the State Pharmacopoeia of the Republic of Kazakhstan [24]. The State Pharmacopoeia of the Republic of Kazakhstan is a set of state standards and regulations governing the quality and safety of medicinal products registered and authorized for use in the established procedure.

Table 2. Organoleptic indicators of tincture.

Indicator	Description
Consistency and appearance	Liquid, homogeneous, transparent without sedimentation.
Taste and odor	Pleasant taste and typical herbal smell
Color	Light brown, uniform across the entire mass

At the same time the content of biologically active substances in tincture is analyzed. The results of the study are presented in Table 3. As can be seen from Table 3, the content of all biologically active substances found in medicinal herbs of the composition has been established. The detected biologically active substances have a positive effect on the immunomodulatory effect of the tincture. Tannins and saponins partially cover the daily rate of an adult.

Table 3. Content of biologically active substances in the tincture.

Name of sample	Indicator	% wt	Daily rate for human, mg
Tincture of Echinacea and Leuzea	Flavonoids	7.9	25
	Tannins	7.5	10
	Saponins	8.3	10

At the next stage of research, the content of amino acids in the resulting tincture was determined. The results of the study are presented in Table 4. As can be seen from Table 4, the obtained tincture contains the highest concentration of the following essential amino acids: histidine, methionine, threonine, phenylalanine, lysine, leucine and isoleucine. As can be seen from Table 5 on safety indicators, the tincture contains heavy metals below the permissible level established by the Technical Regulations of the Customs Union.

Table 4. Essential amino acid content in the tincture of Echinacea and Levsea.

Amino acid	Concentration	WHO, mg per 1 kg of body weight
Histidine	14	10
Mass fraction of leucine and isoleucine	78	59
Lysine	49	30
Methionine	18	10
Cystine	29	5
Phenylalanine	24	25
Threonin	31	15

Table 5. Concentration of heavy metals in tincture.

Indicator	Concentration, mg/100g	Permissible levels of Technical Regulations of the Customs Union
Lead	0.0027	0.5
Cadmium	0.00012	0.03
Arsenic	-	0.05
Mercury	-	0.01

4. Conclusion

Thus, on the basis of the conducted research the formulation from Echinacea and leuzea in an optimum ratio of 1:1 is selected accordingly. The technology of obtaining a tincture from a composition of medicinal herbs is developed and organoleptic, physicochemical, safety indicators, and maintenance of biologically active substances of the tincture are studied. According to the content of biologically active substances and essential amino acids, this tincture can be used in production of fermented milk drinks as a functional ingredient.

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