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SPECTROPHOTOMETRIC QUANTITATIVE DETERMINATION OF FLAVONOIDS AGRIMONIA EUPATORIA L. FROM FLORA OF AZERBAIJAN

The purpose of work was spectrophotometric quantitative determination of flavonoids A. eupatoria L. from flora of Azerbaijan.
As a result, it was revealed that the herb A. eupatoria L., having large stocks of raw materials, growing in the territory of Azerbaijan, contains 0.41% flavonoids.
Key words: *A. eupatoria L., flavonoids, spectrophotometry*

Currently, the demand for plant raw materials in the world market is increasing annually. This demand is associated with a number of affirmative properties of phytopreparations: low toxicity, a wide range of therapeutic action and a small number of side effects. With the identification of new areas of the used species, it is necessary to conduct a comparative pharmacognostic study and establish compliance with the requirements of the monograph. [1].

1 genus of Agrimonia L. grows in Azerbaijan, which has not been studied in the pharmacognostic aspect [2].

Species of the genus Agrimonia L. are studied in pharmacognostic aspect in many countries of the world.

Flavan-3-ols (catechin and procyanidins B1, B2, B3, B6, B7, C1, C2 and epicatechin-epicatechin-catechin), quercetin 3-O-glucoside, quercetin 3-O-galactoside, kaempferol 3-O-glucoside , kaempferol 3-O- (6" - O-p-coumaroyl) -glucoside and quercetin glucosides, as well as various phenolic acids, were obtained and identified in Agrimonia L. [3].

Phenolic acids, amino acids, hydrolyzing tannins and condensed derivatives of ellagic acid and catechins, polysaccharides and volatile oils are present in the herb of A. eupatoria L. [4-6].

The aim of this work was to determine the amount of flavonoids in the herb of A. eupatoria L.

Materials and methods

The raw material of A. eupatoria L. for study was collected in the Gusar region in May-June 2019 in the phase of plant flowering.

Quantitative determination of flavonoids in the raw material of A. eupatoria L. was carried out by spectrophotometric method according to the State Pharmacopoeia XI [7].

Extraction was carried out with 50% ethyl alcohol, 30 ml each, three times; the extracts were combined, transferred to a flask with a thin section with a capacity of 150 ml, and the combined extraction is diluted up to the mark with 50% ethyl alcohol (solution A).

1 ml of the solution A is placed into a 25 ml measuring flask, 2,0 ml 2 % of solution of aluminum chloride is added and the volume of the solution is diluted up to the mark with ethanol 95 % and mixed. After 40 minutes, the optical density of the solution is measured at a spectrophotometer at a wavelength of 415 nm and in a layer thickness 10 mm of cuvette.

In parallel, a standard rutin solution is prepared.

Methodology: An analytical sample of raw materials is crushed to a particle size passing through a sieve with holes 1 mm in diameter. About 1 g (accurately weighed) of the crushed raw material is transferred into a flask with a thin section with a capacity of 150 ml, 30 ml of 50% ethyl alcohol is added, a reflux condenser is connected and heated in a boiling water bath for 30 minutes. After cooling, the extract is filtered into a 100 ml volumetric flask. The extraction is repeated twice under the same conditions with 30 ml of 50% ethyl alcohol for 30 minutes at the second contact of the phases and 30 minutes at the third. The volume of the combined extract is diluted up to the mark with 50% ethyl alcohol (solution A).

1 ml of the solution A is placed into a 25 ml measuring flask, 2,0 ml 2 % of solution of aluminum chloride is added and the volume of the solution is diluted up to the mark with ethanol 95 % and mixed. After 40 minutes, the optical density of the solution is measured at a spectrophotometer at a wavelength of 415 nm and in a layer thickness 10 mm of cuvette.

To prepare a compensation solution, 1 ml of solution A is transferred into a volumetric flask with a capacity of 25 ml, 1 drop of acetic acid is poured and the volume of the solution is diluted to the mark with 95% ethyl alcohol. In parallel, the optical density of the standard rutin solution is determined. The total content of flavonoids in terms of rutin and absolutely dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{D \cdot m_0 \cdot 100 \cdot 100 \cdot 100}{D_0 \cdot m \cdot 100 \cdot (100 - W)},$$

D - the optical density of the test solution; D0 - the optical density of the standard rutin solution; m is the mass of raw materials, g; m0 — mass of standard rutin, g; W - loss in weight during drying of raw materials (%),
for A. eupatoria L. -12.9%.

Preparation of the standard rutin solution

Standard solution - Rutin standard solution is prepared in parallel. 0.05 g of standard rutin is placed in a 100 ml flask, pre-dried at 130-135 ° C for 3 hours, 85 ml of 95% ethanol is added and heated. The resulting solution is cooled, placed in a 100 ml flask, the volume is diluted to the mark with 95% ethanol and mixed.

2.0 ml of 2% aluminum chloride solution is added to the standard solution, the solution volume is diluted to the mark with 95% ethanol and mixed. After 40 minutes, the optical density of the solution is measured on a spectrophotometer at a wavelength of 415 nm and in a 10 mm cuvette [4] (Fig).

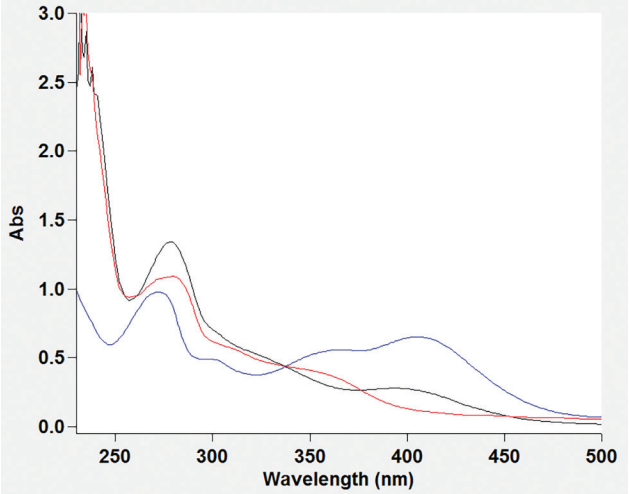

Figure - Absorption spectrum of an alcoholic solution of the herb of A. eupatoria L. and rutin

As a result, it was revealed that the herb of A. eupatoria L., possessing large reserves of raw materials, growing in the territory of Azerbaijan, contains 0.41% of flavonoids.

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СПЕКТРОФОТОМЕТРИЧЕСКОЕ КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ ФЛАВОНОИДОВ AGRİMONİA EUPATORİA L. ИЗ ФЛОРЫ АЗЕРБАЙДЖАНА

Резюме: Целью настоящей работы явилось спектрофотометрическое количественное определение флавоноидов A. eupatoria L. из флоры Азербайджана.
В результате было выявлено, что трава A. eupatoria L., обладающая большими запасами сырья, произрастающая на территории Азербайджана, содержит 0,41% флавоноидов.
Ключевые слова: A. eupatoria L., флавоноиды, спектрофотометрический метод.

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THE CHEMICAL COMPOSITION OF HELIANTHUS ANNUUS L

Resume: *Helianthus annuus L salt, which is necessary for the preparation of natural soap, combines the method of preparation of medical soap production technology. The extract from the stem of the plant. This fresh natural soap contains many useful micro-and macronutrients, biologically active compounds and macro - and micro-elements of the plant Helianthus annuus L were determined by atomic-emission method. Main of them were K (44.9 mg/g), (377.745 mg/g), Na (3.91 mg/g), (7.56 mg/g) and Mg (3.06 mg/g), (1.495 mg/g). These elements are useful for our body and have healing properties. In addition, biologically active substances such as flavonoids, alkaloids, organic acids, coumarins, and vitamins were found in the composition of the plant H. annuus L. These identified data allow us to obtain soap with a high composition and quality and healing properties.*
Key words: *Helianthus annuus L, alkali ash, soap.*

1. Introduction

Nowadays, there are quite a lot of detergents used for different purposes according to their different bactericidal, detergent and other properties, but this does not mean that they fully meet human needs. Because some synthetic detergents have a negative effect on the human body. Moreover, some detergents are made with only one purpose in mind, while some are made for bactericidal purposes, while others have a good washing ability. Therefore, one of the main problems in the industry today is the production of low-cost detergents that have their own properties and do not adversely affect the human body.

Raw materials for general detergents are divided into natural (vegetable and animal oils) and synthetic (petroleum products). Liquid and solid natural oils have long been the initial material of traditional soap in all nations. The properties of soaps obtained by the traditional Kazakh handmade soap method has not yet been extensively studied, and the very narrow area of its application.

The Kazakhs, soap was obtained by mixing and boiling animal fat waste with an alkaline powder from the ashes of chenopodium, artemisia absinthium, haloxylon and other plants. This ash derived alkalis powder is called "Sahar". It uses natural plant extracts that are good for people's health, soap has therapeutic as well as cleansing properties and alleviate skin diseases.

In the work, Helianthus annuus L (sunflower) ashes were used to alkali derivetes. It is easy to find the raw materials around our country. At the same time, its materials are often cheaper and simple preparations. H. annuus L is promising source of a great amount of flavonoids, as well as individual flavonoids that stimulate or inhibit a particular enzymatic process. Phytochemical and pharmacological studies on H. annuus L species demonstrated that many of them are responsible for various biological activities such as cytotoxicity, antioxidant [1], antimalarial [2], moisturizing the skin, nourishing the hair, anti-inflammatory [3,4].

Hence, this present study is a simple procedure for deriving alkali from agricultural waste product such as sunflower wastes and investigating the verifies quantitative, qualitative analysis, macro-, micro elements.

2. Experimental

2.1. Plant material

Plant material H. annuus L was collected in Almaty region, Kazakhstan. The air dried part of H. annuus L was cutted into small pieces and stored at room temperature until its usage.

2.2. Method of obtaining Sahar from H. annuus L

750 ml of water was added to 100 g ash (Fig.1) obtained by burning H. annuus L in a conical flask, which was kept at the temperature of 100°C for 30 minutes boiled continuously mixing the mixture. After that, the ash content was filtered through the filter paper by using vacuum pump filtration. The filtrate is boiled by aluminum container until the water is drawned, white salt is removed at the bottom of the dish. The resulting alkali salt was dried, crushed, and a white crystalline sahar is obtained (Fig. 2).

2.3.The quantitative and qualitative analysis

Bioactive constituents of H. annuus L were determined by according to methods reported in the monograph [5]. The content of extractives in H. annuus L has been determined by 80% ethanol solutions in water in accordance with method reported in the State Pharmacopeia X [6].

2.4.The determination of macro, microelements

The contents of major elements of alkali ashes were determined by Shimadzu 6200 series spectrometer. 2.52 g of raw material was placed in a pre-calcined and accurately weighted porcelain crucible. Then the crucible was gently heated, first letting the substance burn at the lowest possible temperature, and the flame was gradually increased. Calcination was performed at 500°C to obtain a constant mass. At the end of the calcination, the crucible was cooled in a des-

iccator and then the resulting ash was burned again at 600°C until a uniform gray color was obtained. The ash of H. annuus L (0.199 g) was dissolved in 10.0 mL of 40% nitric acid by heating. After that, the resulting solution was heated to obtain wet salts. Subsequently, it was dissolved in 15.0 mL of 1 N nitric acid and transferred to a 25.0 mL volumetric flask for analysis.

3. Results and discussion

Quantitative and qualitative analysis of bioactive constituents, moisture content, total ash and extractives of H. annuus L are given in Table 1.

Table 1 – Quantitative analysis of biologically active constituents of H. annuus L

Component	Content, %
Moisture	5.8
Ash	9.3
Extractives	4.88
Alkaloids	2.07
Flavonoids	0.76
Polysaccharides	1.49
Coumarins	0.05
Organic acids	1.13
Vitamin B2	0.028
Vitamin C	0.056

Moisture and ash content vary within certain limits for every plant and depends on the nature of the plant material itself, how it is collected and dried. For H. annuus L the limit is 12 %, according to the State Pharmacopeia X [6]. Therefore, the determination of these contents was necessary to prove the good quality of H. annuus L.

The largest quantity of extractives in H. annuus L was obtained with 80% alcohol. Thus, this appropriate solvent could be utilized in the extraction. The identification of extractive substances by an appropriate solvent is important as it determines the good quality of plant for the content of biological metabolites.

Most flavonoids have multiple anti-aging effects on the skin, blood vessels, and immune cells, which are immediately perceived by the naked eye. This action includes controlling inflammation, protecting against damage caused by free radicals, strengthening blood vessels, inhibiting fibrosis, protecting collagen and elastin from degradation, repairing the skin, stimulating the synthesis of new collagen and elastin, etc.[7].

As regards alkaloids, they are important secondary metabolites that are known to possess therapeutic properties. Moreover able to prevent the onset of various degenerative diseases by free radical scavenging or binding with the oxidative reaction catalyst [8]. Organic acids are responsible for the taste, the flavour, the microbial stability, and the product consistence of plant derived beverages and are used in food preservation because of their effects on bacteria.

Polysaccharides are unique substances that can retain moisture in the dermis, keep the skin elastic, stimulate the synthesis of collagen fibers, and improve the immunity of cells.

Vitamine C has the ability to increase the amount of collagen and maintain healthy blood circulation, so they are ideal for athletes who adhere to natural supplements.

In medicine, coumarin is used as an antispasmodic and a means to prevent the formation of blood clots. Coumarin regulates the blood clotting factor. Pharmacists use coumarin as a masking aromatic element in the production of medicines.

Figure 1 - The ash of H.annuus L

Figure 2 - Sahar of H. annuus L

Composition of macro-micro elements in the ash and Sahar of H. annuus L were showed in Table 2.

Nine macro-, micro elements were obtained from the ash of plant. Main of them were K (44.9mg/g), Na (3.91 mg/g) and Mg (3.06mg/g) as shown in Table 2. It appears that H. annuus L is a great source of macro- and micro elements, which are necessary for the functioning of the muscular, cardiovascular, immune, nervous systems and

Table 2 – Composition of macro-micro elements in the ash and Sahar of H. annuus L

	H. annuus L		
Element	**Concentration in ash, mg/g**	**Concentration in plant, mg/g**	**Concentration in Sahar, mg/g**
Ca	80.0	7.44	0.006
K	44.9	4.17	377.745
Mg	3.06	0.284	1.495
Na	3.91	0.364	7.56
Fe	0.240	0.022	0.010
Mn	0.006	0.001	0.0014
Zn	0.189	0.018	0.005
Cu	0.185	0.017	0.0014
Ni	0.017	0.002	0.0072

participate in the synthesis of vital compounds, metabolic processes, blood formation, digestion and neutralization of metabolic products. Magnesium is involved in many processes occurring in the body - in energy production,nerve signal transmission, protein synthesis, bone construction, regulation of relaxation and tension of blood vessels and muscles. It has a calming effect, reducing the excitability of the nervous system and enhancing the processes of inhibition in the cerebral cortex, acts as an anti-allergic and anti-inflammatory factor, protects the body from infection, participating in the production of antibodies, plays a significant role in the processes of blood clotting [9]. Together with potassium, sodium performs the following functions: creating conditions for the emergence of the membrane potential and muscle contractions, maintaining the osmotic concentration of the blood, maintaining the acid-base balance, normalization of the water balance, provision of membrane transport, activation of many enzymes [10]. Potassium ions provide transport of CO2, and calcium ions regulate the permeability of cell membranes. Potassium is able to suppress the uncontrolled growth of skin cells in psoriasis [11]. Calcium is involved in the transmission of nerve impulses, provides balance between the processes of excitation and inhibition in the cerebral cortex, participates in the regulation of contractility of skeletal muscles and heart muscles, affects the acid-base balance of the body [12].

Sahar contains many useful micro- and macro elements.The main ones are. Main of them were K (377.745 mg/g), Na (7.56 mg/g) and Mg (1.495 mg/g) as shown in Table 2. The total weight of the resulting sahar is 23 grams, this is 23%.Sahar is prepared for the treatment of skin diseases with the addition of other additives. It is a cure for infectious skin diseases.

Conclusions

This research demonstrated total biologically active components, nine macro-micro elements of H. annuus L, as well as constituent importance of these substances. According to the results of the present study, it has been proven that this plant is one of the valuable sources of alkaloids, flavonoids, macro-micro elements, such as K, Mg, Na and Ca. Thus, it is logical to conclude that presence of these bioactive constituents in H. annuus L may indicate that the plant contains substances capable to prevent soap made from sahar is affordable and it is a cure for many skin diseases. It has many benefits, such as skin hydration, hair nutrition, anti-inflammatory, disinfecting, suppressing swelling, reducing pain. It is used for headaches, psoriasis, itching of the skin, natoptysh, boils, acne, rheumatism, ear diseases, hemorrhoids and other diseases.

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HELIANTHUS ANNUUS L-ДЫҢ ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ

Түйін: Табиғи сабын жасау үшін қажет Helianthus annuus l тұзы медициналық сабын жасау әдісін өндіріс технологиясымен біріктіреді. Ол өсімдіктің сабағынан алынған сығынды. Бұл жаңа табиғи сабын құрамындағы көптеген пайдалы микро және макроэлементтер, биоактивті қосылыстар және Helianthus annuus L өсімдіктерінің макро-және микроэлементтері атомды-эмиссионды әдіспен анықталды. Олардың негізгілері K (44,9 мг/г), (377,745 мг/г), Na (3,91 мг/г), (7,56 мг/г) және Mg (3,06 мг/г), (1,495 мг/г) болды. Бұл элементтер біздің ағзамызға пайдалы және емдік қасиеттерге ие. Сонымен қатар, H. annuus L. өсімдігінен флавоноидтар, алкалоидтар, органикалық қышқылдар, кумариндер және дәрумендер сияқты биологиялық белсенді заттар анықталды. Бұл анықталған деректер құрамы мен сапасы жоғары, сондай-ақ емдік қасиеттері бар сабын алуға мүмкіндік береді.
Түйінді сөздер: Helianthus annuus L, сілтілі тұз, сабын.

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ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА HELIANTHUS ANNUUS L

Резюме: Согласно литературным данным, подсолнечное масло богато полезными веществами, однако, в зависимости от степени очистки масла, количество этих компонентов варьируется. В данном исследовании был изучен дистиллят жирных кислот подсолнечного масла. Согласно полученным данным, более 40% состава кислот приходится на линолевую кислоту с F-витаминной активностью. В большом количестве содержатся стерины, составляющие 44% неомыляемого остатка. Токоферолы составляют 9% неомыляемого остатка, 93% из них приходится на а-токоферол. Helianthus annuus L необходим для приготовления натурального мыла, сочетает в себе способ приготовления медицинского мыла с технологией производства. Экстракт из стебля растения. Это свежее натуральное мыло содержит много полезных микро-и макроэлементов, биологически активные соединения и макро - и микроэлементы растения Helianthus annuus L были определены атомно-эмиссионным методом. Основными из них были K (44,9 мг/г), (377,745 мг/г), Na (3,91 мг/г), (7,56 мг/г) и Mg (3,06 мг/г), (1,495 мг/г). Эти элементы полезны для нашего организма и обладают целебными свойствами. Кроме того, в составе растения H. Annuus L. были определены биологически активные вещества, такие как флавоноиды, алкалоиды, органические кислоты, кумарины и витамины. Эти выявленные данные позволяют получать мыло с высоким качеством, а также целебными свойствами.
Ключевые слова: Helianthus annuus L, щелочная зола, мыло