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STUDY OF TANNING SUBSTANCES AND FLAVANOIDS IN THE LEAVES OF ASIAN AGRIMONY *(AGRIMONIA ASIATICA* JUZ.)

Asian agrimony *(Agrimonia asiatica* Juz) is a common wild plant including that growing within the territory of Kazakhstan, a very promising raw material for flavonoids and tanning substances release. These active substances have significant pharmacological potential. The article presents the results of work on determining the quantitative and qualitative composition of tannins and flavonoids in plant raw materials from the Asian agrimony *(Agrimonia asiatica* Juz). The presence of tanning substances and flavonoids in the extracts received from the above-ground part of Asian agrimony has been established by qualitative reactions. Various developers for paper chromatography have been used. The quantitative determination of flavonoids has been carried out in terms of the equivalent amount of quercetine, and the standard method of permanganatometric titration has been used to determine the quantitative content of tanning substances. Quantitative content of tanning substances in the leaves of Asian agrimony *(A. asiatica juz.)* showed an 8,156 %, and a quantitative content of flavonoids showed 1,9 %. The purpose of the study is to determine the quantitative and qualitative composition of tannins and flavanoids in prepared herbal medicinal raw materials in order to identify the most promising objects for further research.

Key words: Asian agrimony *(Agrimonia asiatica Juz),* tanning substances, flavonoids, phytochemistry.

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Азия ошағаны (*Agrimonia asiatica* Juz) жапырағындағы илік заттар мен флаваноидтарды анықтау

Азиялық ошаған (*Agrimonia asiatica* Juz) кең таралған жабайы өсімдік. Ол Қазақстан аумағында өсетін өсімдіктер ішіндегі флавоноидтар мен илік заттарды бөліп алу үшін өте маңызды шикізат болып табылады. Бұл белсенді заттар үлкен фармакологиялық қасиетке ие.

Флавоноидтар және илік заттар барлық өсімдіктер құрамында кездеседі. Олар антигепатоксикалық, антибактериалық, антимутагендік, әртүрлі саңырауқұлақтарға қарсы сондай-ақ қанның қалпына келуіне, суық тигенге қарсы белсенділігі бары анықталған.

Мақалада азиялық ошағанның (*Agrimonia asiatica* Juz) өсімдік шикізатындағы илік заттар мен флавоноидтардың сандық және сапалық құрамын анықтау жұмыстарының нәтижелері берілген. Азиялық ошағанның жер үсті бөлігінен алынған сулы сығындыларда илік заттар мен флавоноидтардың болуы сапалық реакциялар арқылы анықталды. Қағаз хроматографиясы үшін әртүрлі айқындауыштар пайдаланылды.

Флавоноидтарды сандық анықтау кверцетин бойынша жүргізілді, ал таниндердің сандық құрамын анықтау үшін перманганатометриялық титрлеудің стандартты әдісі қолданылды. Азиялық ошағанның (*A.asiatica* Juz.) жапырақтарындағы илік заттардың сандық мөлшері 8,156 %, ал флавоноидтардың сандық құрамы 1,9 % болды.

Біздің жұмысымыздың мақсаты –әрі қарай зерттеу үшін перспективалық объектілерді анықтау мақсатында дайын шөптік дәрілік шикізаттардағы илік заттар мен флавоноидтардың сандық және сапалық құрамын анықтау.

Түйін сөздер: Азиялық ошаған (*Agrimonia asiatica* Juz), илік заттар, флавоноидтар, фитохимия.

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Изучение дубильных веществ и флаваноидов в листьях репейника азиатского (Agrimonia asiatica Juz.)

Репейник азиатский (*Agrimonia asiatica* Juz) является распространенным дикорастущим растением. Это очень важное сырье для извлечения флавоноидов и питательных веществ из растений, произрастающих в Казахстане. Эти активные вещества обладают прекрасными фармакологическими свойствами.

Флавоноиды и дубильные вещества содержатся во всех растениях. Обладают антигепатотоксической, антибактериальной, антимутагенной, противогрибковой активностью, а также кровеостанавливающей, противопростудной активностью.

В статье представлены результаты работ по определению количественного и качественнного состава дубильных веществ и флавоноидов в растительном сырье из репейника азиатского (*Agrimonia asiatica* Juz). Наличие дубильных веществ и флавоноидов в полученных водных извлечениях из надземной части репейника азиатского установили качественными реакциями. Использовались различные проявители для бумажной хроматографии.

Количественное определение флавоноидов проводили в пересчете на кверцетин, а для определения количественного содержания дубильных веществ использовали стандартную методику перманганатометрического титрования. Количественное содержание дубильных веществ листьях репейника азиатского *(A. asiatica* Juz.*)* показало 8,156 %, а количественное содержание флавоноидов показало 1,9%.

Цель исследования — проведение определения количественного и качественнного состава дубильных веществ и флаваноидов в готовом растительном лекарственном сырье для выявления наиболее перспективных объектов для дальнейших исследований.

Ключевые слова: Репейник азиатский (*Agrimonia asiatica* Juz), дубильные вещества, флавоноиды, фитохимия.

Introduction

Medicinal plants are of great importance for the health of people and the community. The medicinal value of the plant is in some chemicals that have a certain physiological effect on humans. The most important of these biologically active plant components are alkaloids, tanning substances, flavonoids, and phenolic compounds. Many medicinal plants are used as food spices. They are also sometimes added to the products intended for pregnant and nursing mothers for medicinal purposes [1, 2].

Among the various classes of plant compounds that determine their therapeutic effect, a significant place is occupied by flavonoids, which constitute a powerful antioxidant system in plants that can restore oxidized forms of antioxidant compounds [3, 4].

Flavonoids are related to the class of natural polyphenol compounds. They are very common in nature. Flavonoids contain two phenyl waste products. These phenyl waste products are chained to internal atoms of carbon. A five-membered or six-membered oxygen-forming heterocycle is formed and closely connected to flavonoids, they are connected by a benzene nucleus [5,6].

Flavonoids are phytochemicals present in many plants, fruits, vegetables, and leaves, with potential application in medicinal chemistry [7].

Recent studies carried out by scientists have been devoted to the study of the medicinal qualities of flavonoids. Many flavonoids have been shown to have antioxidant activity, hepatoprotective, prevent coronary heart disease, the ability to capture free radicals, anti-inflammatory and antitumor activity while some flavonoids exhibit potential antiviral activity [8]. In plant systems, flavonoids help to push back against oxidative stress and to act as growth regulators. For pharmaceutical purposes, the cost-effective mass production of various types of flavonoids using microbial biotechnology has become possible [9].

Most of all flavonoids are found in actively functioning organs: leaves, flowers, fruits (color, aroma), seedlings, as well as in integumentary tissues that perform protective functions. Different organs and tissues differ not only in quantity, but also in the qualitative composition of flavonoids. [10, 11].

One of the common flavonoids is luteolin, and its glycosides are widespread in plants. The ability of luteolin to prevent carcinogenesis in animal mod-

els, induce apoptosis, inhibit angiogenesis, reduce tumor growth *in vivo.* They increase the sensitivity of tumor cells to the cytotoxic effects of certain antitumor drugs suggests. This flavonoid has cancer chemotherapeutic and chemoprophylaxis potential [12].

The term "tanning substances" shall be understood as a specific "tanning" effect of organic substances mostly of a polyphenol nature [13].

Almost all plants contain tanning substances of hydrolyzable and condensate or mixed types.

In plants, hydrolysable and condensed tannins occur simultaneously, with the predominance of one class. The accumulation and composition of tannins can be influenced by the age and phase of plant development, climate, soil conditions, altitude factor, lighting, humidity, collection time and drying methods [14, 15].

Some scientific data have established that silt substances have antihepatotoxic, antibacterial, antimutagenic, antifungal activity against various fungi, and they also promote blood regeneration, have anti-cold activity [16].

The therapeutic potential of the tanning substances is connected with a phenolic hydroxyl group present on the surface of the tanning substances. This group binds to protein adhesins and contributes to inhibition of enzymes, rupture of plasma membrane and microbial substrate deprivation. Due to their antiseptic properties, the tanning substances are currently being tested against pathogenic organisms [17, 18].

The active substances that have phenolic groups in their structure have significant pharmacological potential. Pharmacological studies have established the presence of anti-inflammatory, antimicrobial and antioxidant activity in Asian agrimony *(A. asiatica juz)* [19, 20].

Further pharmacognostic study of the herb Asian agrimony is an urgent problem and will expand the domestic raw material base of medicinal plant materials, as well as create prerequisites for further in-depth study in order to introduce a new type of raw material into scientific medicine.

Thus, the purpose of the study of our work was to determine the quantitative and qualitative composition of tannins and flavanoids in herbal medicinal raw materials in order to identify the most promising objects for further research.

Materials and Methods

The object of the research was the leaves of the Asian agrimony *(Argimonia asiatica)* that have been collected 40 km from southeast Kazakhstan east of Almaty in the foothills of Zailiyskiy Alatau. Dried and crushed leaves have been used (crushed to 2-5 mm). Drying of the studied samples was carried out in the open air in the shade in accordance with the requirements of the SP RK. The finished raw materials were placed in paper bags and stored in accordance with the requirements of the general pharmacopoeial article OFS.1.1.0011.15 SP XIIIth edition, v. 1 "Storage of medicinal plant materials and herbal medicinal preparations" in a dry, clean, well-ventilated room. [22].

Qualitative determination of flavonoids

Flavonoids from the aqueous phases are extracted sequentially using ether (aglycones), ethyl acetate (monosides) and butanol (biosides, triosides, etc.)

Qualitative assessment was carried out by methods of one- and two-dimensional chromatography. To determine the substance, it is important to determine the value of Rf. The definition of the Rf value is the ratio of the distance from the spot center to the application point to the distance from the application point to the solvent front.

When carrying out the cyanidin reaction, a pink color is observed. The appearance of a yellow-green color when 1% aluminum chloride solution is added to the extract under study, the appearance of a yellow-brown color with an ammonia solution confirm the presence of flavonoids in the Asian burdock leaf. Thus, the results of qualitative reactions indicate the presence of flavonoid glycosides in the studied plant [23].

Quantitative determination of flavonoids (in terms of the equivalent amount of quercetin)

For analysis, the raw material derived from the leaves of Asian agrimony was cut to small particles in order to pass through a sieve with a diameter of 1 mm.

1 g of raw material derived from the leaves of agrimony was placed in a 150 ml flask, 30 ml of 90% alcohol containing 1% concentrated hydrochloric acid was added. Then the flask was connected to a reflux condenser then heated in a boiling water bath for 30 minutes. Then the flask was cooled to room temperature and extract was filtered through a paper filter into a 100 ml flask. The extraction was repeated 2 more times in the manner specified above. The extracts were mixed and filtered through a filter into the same volumetric flask. Then filter was washed using 90% alcohol, the filtrate was made up to the volume using 90% alcohol. This is our solution A. 2

ml of solution A were placed in a 25 ml volumetric flask, 1 ml of 1% solution of aluminum chloride in 95% alcohol was added, and it was made up to the volume. After 20 minutes, the optical density of the solution on a spectrophotometer Apel-303 (Japan) at the wavelength of 430 nm was measured. We used cuvettes with a layer thickness of 10 mm [22].

For comparison, we used 2 ml of solution A made up to the volume using 95% alcohol in a 25 ml volumetric flask. Control experience. Add 25 ml of indigo sulfonic acid to 525 ml of water and titrate with potassium permanganate solution until golden yellow.

For comparison, we used 2 ml of solution A made up to the volume using 95% alcohol in a 25 ml volumetric flask.

Dry raw materials in percentage points (X), the total content of flavonoids in terms of the equivalent amount of quercetin are calculated by the formula:

$$X= (D*25*100*100)/764,6*m*2*(100-W)$$

where, D – optical density of the test solution at the wavelength of 430 nm; 764.6 – specific absorbance of quercetin complex with 1% aluminium chloride solution at the wavelength of 430 nm; m – mass of raw materials in grams; W – loss in mass during drying of raw materials in percentage [23].

Qualitative determination of tanning substances in plant raw materials

Precipitation ofindicatehous precipitate, soluble in excess reagent, when adding freshly prepared 1% gelatin solution and 10% hydrochloric acid solution to the aqueous extract from Asiatic agrimony leaves indicates the presence of tannins. When formaldehyde solution of 40% and hydrochloric acid as well as bromine water solution of 0.5% are added to the test extract, no precipitation is formed. The precipitation of white when added to the aqueous extraction solution of medium lead acetate 2% indicates the presence of tannins of the hydrolysable group. The appearance of a dark blue colouring when mohr's salt (MS) 1% solution is added to the water extract confirms that tannins of the hydrolysable group predominate in the leaf of *Asian agrimony.*

Quantitative determination of tanning substances in plant raw materials

To determine the quantitative content of tanning substances, the standard method of permanganatometric titration proposed by State Pharmacopoeia of the Republic of Kazakhstan has been used. T.1. Determination of the tanning substances content in medicinal plant raw materials in terms of the equivalent amount of tannin

2 g of cut raw material derived from the leaves of Asian agrimony and sieved through a sieve with a diameter of 3 mm was placed in a 500 ml conical flask. Then 250 ml of boiled water was poured and boiled at reflux for 30 min with periodic stirring. Then, 25 ml of the resulting extract was pipetted into other conical flask. 500 ml of water, 25 ml of indigosulfo acid were added and titrated with constant stirring until gold color using a solution of potassium permanganate (0.02 mol / L) [22].

At the same time, a control experiment is carried out. As a control, a solution is used where distilled water is used instead of an aliquot of the sample. 1 ml of potassium permanganate solution corresponds to 0.004157 g of tanning substances in terms of the equivalent amount of tannin.

The content of tanning substances (X) in percentage in terms of the equivalent amount of dry raw materials is calculated using a special formula [23]. For comparison, we used 2 ml of solution A made up to the volume using 95% alcohol in a 25 ml volumetric flask.

Dry raw materials in percentage points (X), the total content of flavonoids in terms of the equivalent amount of quercetin are calculated by the formula:

$$X=(D*25*100*100)/764,6*m*2*(100-W)$$

where, D – optical density of the test solution at the wavelength of 430 nm; 764.6 – specific absorbance of quercetin complex with 1% aluminium chloride solution at the wavelength of 430 nm; m – mass of raw materials in grams; W – loss in mass during drying of raw materials in percentage

The experiments were carried out in 3 speeds and the error of the mean value was calculated in accordance with the requirements of the SP of the Republic of Kazakhstan. [23].

Results and discussion

Determination of quantitative and qualitative composition of tanning substances in plant raw materials

As a result of qualitative reactions to extraction of the grass of Asian agrimony, the presence of tanning substances has been established.

The presence of the tanning substances in the extracts received from the above-ground part of Asian agrimony has been established by qualitative reactions specified in Table 1. Different developing chemicals have been used for paper chromatography.

Table 1 – Qualitative content of tannins in the leaves Asian agrimony *(A. asiatica* Juz.)

№	Different developing chemicals	Substances	Reaction
1	1% aqueous or aqueous-alcohol solution of Mohr's salt (MS) 1% aqueous or water-alcohol solution of Mohr's salt (MS)	Tanning substances	**Positive** (black and green coloring)
2	2% lead acetate solution	Tanning substances	**Positive** (white precipitate

Table 2 – Quantitative compound of tannins in the leaves of Asian agrimony *(A. asiatica* Juz.)

№	Quantitative compound of tanning substances, %
1	8,157± 0,38
2	8,155± 0,41
3	8,156± 0,39
Average	8,156± 0,39

According to the tables 2 it follows that the quantitative content of the tanning substances in the leaves of Asian agrimony *(A. asiatica juz.)* showed 8,156± 0,39%. This means that tanning substances in the leaves of Asian agrimony *(A. asiatica juz.)* are contained within the permissible values for medicinal plant raw materials.

Determination of quantitative and qualitative composition of flavonoids in plant raw materials

The presence of flavonoids in the alcohol (50% ethyl alcohol) extracts received from the aboveground part of Asian agrimony has been established by the qualitative reactions given in Table 3.

Table 3 – Qualitative content of flavonoids in leaves Asian agrimony *(A. asiatica* Juz.)

№	Different developing chemicals	Substances	Reaction
1	*1% solution of aluminum chloride*	Flavonoids	**Positive** (Yellow)
2	*Ammonic solution*	Flavonoids	**Positive** (Yellow)

The results of qualitative reactions indicate the presence of flavonoids in the leaf of Asian agrimony. When cyanidine reaction is carried out, a pink staining is observed. The appearance of yellow-green coloring when adding aluminum chloride 1% solution to the test extraction, the appearance of yellow-brown coloring with ammonia solution confirms the presence of flavonoids in the leaf of Asian agrimony. Thus, the results of qualitative reactions indicate the presence of flavonoid glycosides in the studied plant.

Quantitative determination of flavonoids has been carried out in terms of the equivalent amount of quercetin

Table 4 – Quantitative compound of flavonoids in the leaves of Asian agrimony *(A. asiatica* Juz.)

№	Quantitative compound of flavonoids, %
1	1,903± 0,29
2	1,902± 0,27
3	1,904± 0,31
Average	1,903± 0,29

For the quantitative determination of flavonoids, was used a spectrophotometric method. It is based on the reaction of interaction of flavonoids with aluminum chloride in the environment of 70% ethyl alcohol. Table 4 shows the average number of flavonoids in the leaves of Asian agrimony *(A. asiatica* juz.). The quantitative content of flavonoids showed 1,903± 0,29.

As a result of the experiments, the quantitative and qualitative determination of tanning substances and flavonoids in the leaves of Asian agrimony was carried out.

Discussion

Phytochemical study of the tanning substances and flavonoids in the plant raw materials of Asian agrimony showed that the leaves were rich in flavonoids, tanning substances. They were known for their medicinal activity, and they also exhibit physiological activity. The plant studied may be considered as a potential source of healthful drugs. In many literature sources, according to the description of medicinal plants, the content of tanning sub-

stances was noted on average of 0.3-4%, rarely – up to 34% [24]. In the course of the study performed, the tanning substances of Asian agrimony showed 8,156± 0,39%, and the quantitative content of flavonoids showed an average of 1,903± 0,29%.

From these results, we can conclude that the polyphenol, flavonoid and tannin content varies according to the, geographical (or geomorphological, altitude, latitude, type of relief) and climatic conditions (temperature, rainfall, humidity) of the locality where the leaves are collected, and the type of extract (organic or aqueous) [25].

It is known that the content of biologically active substances in plants depends on the phase of vegetation. Scientists have found that the maximum content of flavonoids is observed in the above-ground organs during the flowering phase. Based on these results, we carried out the determination of tannins and flavonoids in the aboveground organs of Asian agrimony [26].

One of the most common groups of natural phenolic compounds are flavonoids, since almost all plants contain them in greater or lesser amounts. Many of them are plant pigments and protect tissues from the harmful effects of ultraviolet rays by acting as filters. They are involved in plant respiration and together with ascorbic acid in redox processes. Because of their high pharmacotherapeutic activity and low toxicity even with prolonged use, flavonoids are widely used in various branches of medicine and pharmacy. As a group of biologically active substances, they have anti-inflammatory, capillary-strengthening, P-vitamin, antioxidant properties and exhibit antitumor, antimicrobial and diuretic activity. Medicinal plant raw materials containing flavonoids are used in medical practice as a source of antispasmodic, choleretic, hepatoprotective, anti-ulcer, antioxidant, anti-inflammatory, angioprotective and other medicines. In addition, flavonoid preparations exhibit pharmacological activity in cardiovascular pathology.

The content of total flavonoids and antioxidants in the aerial part of S. sub dentata of the flora of Dagestan ranges from 1.9 to 2.9% and from 4.3 to 7.9 mg/g in terms of air-dry raw materials, respectively. At the same time, a slight decrease in the total content of antioxidants in the aboveground part of the species was noted in introduced samples compared to natural ones [27].

In many literature sources, according to the description of medicinal plants, the content of tannins is noted, averaging 0.3-4%, rarely up to 34% (Potentilla erecta (L.) Raeusch) [5]. In the course of the study, the following results were obtained (%): oak bark 16%, crushed St. John's wort leaves 10.4%, rhizomes (13%) and burnet roots (18.5%).

The tannins of the Siberian geranium were studied and their content in the rhizomes is 20.8-30.0%, and the content of tannin in the leaves is 20.3%.

The study of the quantitative content of tannins in the aerial organs of the Daurian rhododendron showed that most of all tannins accumulate in the leaves, flowers and shoots of the current year, in lignified shoots their content decreases by almost 3 times [28].

In terms of the quantitative content of flavonoids and tannins, the aerial organs of the studied plant are unequal, most of all these compounds accumulate in the leaves and stems of the current year, and their maximum content falls on the phases of the beginning and mass ripening of fruits. In these terms it is recommended to carry out the procurement of raw materials (shoots of the current year) of Asian burdock.

Conclusion

Based on the results obtained with respect to quantitative determination of flavonoids and tanning substances, the use of the extract derived from *Agrimonia asiatica* plant raw materials for treatment of the diseases such as inflammation, hepatitis and wounds may be recommended.

Based on the obtained data for determining the quantitative content of tannins, we can specifically talk about the use of the studied plants in the treatment of a number of diseases that require the presence of tannins as bactericidal, astringent, hemostatic agents in the form of extracts.

The study of the dynamics of tannins and flavonoids in the vegetative organs of Agrimonia asiatica makes it possible to recommend young shoots 12–15 cm long as a medicinal plant material.

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