PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

PRODUÇÃO DE FRAÇÕES DE MIRICETINA DE PLANTA DE *LINOSYRIS VILLOSA* E ESTUDO DE SUA ATIVIDADE BIOLÓGICA

ISOLATION OF MIRICETINE-CONTAINING FRACTIONS FROM *LINOSYRIS VILLOSA* PLANT AND THEIR APPLICATION AS ANTIANEMIC AGENT

ВЫДЕЛЕНИЕ МИРИЦЕТИНСОДЕРЖАЩИХ ФРАКЦИЙ ИЗ РАСТЕНИЯ *LINOSYRIS VILLOSA* И ИХ ПРИМЕНЕНИЕ В КАЧЕСТВЕ АНТИАНЕМИЧЕСКОГО СРЕДСТВА

NAZAROVA, Valentina D.[1]; SALIKOVA, Natalya S.[2*]; BEKTEMISSOVA, Ainash U.[3]; BEGENOVA, Bahyt E.[4]; AUBAKIROVA, Gulsim B.[5];

[1,3,4,5] M. Kozybayev North Kazakhstan State University; Department of Chemistry and Chemical Technology; 86 Pushkin Str.; ZIP code 150000; Petropavlovsk – Republic of Kazakhstan.

[2] Abay Myrzakhmetov Kokshetau University; Department of Ecology, Life Safety, and Environmental Protection; 189A Auezov Str.; ZIP code 020000; Kokshetau – Republic of Kazakhstan.

** Correspondence author*
e-mail: natsal66@mail.ru

Received 22 March 2020; received in revised form 06 June 2020; accepted 03 July 2020

RESUMO

Com o desenvolvimento da química, substâncias naturais foram ativamente substituídas da vida humana por produtos químicos. No entanto, apesar do enorme progresso nessa área, nas últimas décadas, os problemas de graves efeitos colaterais dos produtos químicos sintéticos no meio ambiente tornam-se cada vez mais pronunciados. Foram utilizados os métodos de cromatografia bidimensional e unidimensional de papel, cromatografia de adsorção em coluna, cromatografia gasosa e espectroscopia de infravermelho. O objetivo do artigo é estudar a composição química da planta de *Linosyrisv villosa* por métodos físico-químicos de análise e criar formas de dosagem baseadas nela. O problema do artigo é a busca e criação de medicamentos baseados na flora do norte do Cazaquistão. A novidade científica reside no fato de que a composição química e a atividade biológica da planta *Linosyris villosa*, que cresce no território do norte do Cazaquistão, estão sendo estudadas pela primeira vez. De acordo com os resultados da análise, em 13% dos pacientes o efeito do tratamento é insignificante e em 87% dos pacientes houve uma melhoria nas contagens sanguíneas (aumento da hemoglobina, aumento no número de glóbulos vermelhos, quase todos os pacientes retornaram a contagem normal de cores), o número de leucócitos aumentou para normal e a fórmula de leucócitos melhorou. Portanto, o medicamento “Vitin”, que é um extrato de água-álcool da planta *Linosyris villosa*, é um medicamento eficaz para o tratamento de pacientes com anemia de várias gravidades. O significado prático do trabalho é definido da seguinte forma: os compostos biologicamente ativos da planta *Linosyris villosa* foram obtidos com o objetivo de estudá-los e aplicá-los na medicina e na agricultura.

Palavras-chave: *planta de Linosyris villosa, flavonóides, frações de miricetina, filmes de drogas, atividade antianêmica, pesticidas.*

ABSTRACT

With the development of chemistry, natural substances were actively supplanted from human life by chemical products. However, despite the enormous progress in this area, in recent decades, the problems of serious side effects of synthetic chemicals on the environment have become more and more pronounced. Methods of two-dimensional and one-dimensional paper chromatography, column adsorption chromatography, gas chromatography, and IR spectroscopy were used. The purpose of the article is to study the chemical composition of the *Linosyris villosa* plant by physicochemical methods of analysis. Creation of dosage forms based on it. The problem of the article is the search and creation of drugs based on the flora of Northern Kazakhstan. The scientific novelty lies in the fact that the chemical composition and biological activity of the *Linosyris villosa* plant, growing in the territory of Northern Kazakhstan, is being studied for the first time. According to the results of the analysis, in 13% of patients the treatment effect is insignificant, and in 87% of patients there was an improvement in blood values (increase in hemoglobin, an increase in the number of red blood cells, almost

DOI: 10.52571/PTQ.v17.n35.2020.82_NAZAROVA_pgs_998_1012.pdf

all patients returned color index of blood), the number of white blood cells increased to normal, and the WBC differential improved. Therefore, the drug “Vitin”, an aqueous-alcoholic extract of the *Linosyris villosa*, is an effective drug for the treatment of patients with anemia of varying severity. The practical significance of the work is defined as follows: biologically active compounds from the *Linosyris villosa* plant were obtained for the purpose of studying and applying them in medicine and agriculture.

Keywords: *Linosyris villosa* plant, flavonoids, myricetin fractions, medicated films, antianemic activity, pesticides.

АННОТАЦИЯ

С развитием химии, природные вещества активно вытеснялись из жизни человека продуктами химического производства. Однако, несмотря на огромный прогресс в этой области, в последние десятилетия все настоятельнее заявляют о себе проблемы серьезных побочных воздействий синтетических химических препаратов на окружающую среду. Были использованы методы двумерной и одномерной бумажной хроматографии, адсорбционной хроматографии на колонках, газовая хроматография, ИК-спектроскопия. Целью статьи является изучение химического состава растения *Linosyrisv villosa* физико-химическими методами анализа. Создание на его основе лекарственных форм. Проблемой статьи является поиск и создание лекарственных препаратов на основе флоры Северного Казахстана. Научная новизна заключается в том, что химический состав и биологическая активность растения *Linosyris villosa,* произрастающего на территории Северного Казахстана, изучается впервые. По результатам анализа у 13% больных эффект лечения незначительный, а у 87% больных отмечалось улучшение показателей крови (увеличение гемоглобина прирост количества эритроцитов, почти у всех больных нормализовался цветной показатель крови), увеличилось количество лейкоцитов до нормы, улучшилась лейкоцитарная формула. Следовательно, препарат «Витин», представляющий собой водно-спиртовой экстракт растения *Linosyris villosa*, является эффективным препаратом для лечения больных с анемией различной тяжести. Практическая значимость работы определяется в следующем: получены биологически активные соединения из растения *Linosyris villosa* с целью изучения и применения их в медицине и сельском хозяйстве.

Ключевые слова: *растение Linosyris villosa, флавоноиды, мирицетиновые фракции, лекарственные пленки, антианемическая активность, пестициды*.

1. INTRODUCTION

A person uses plants not only as food but also as therapeutic agents, as well as in perfumes and as dyes. With the development of chemistry, natural substances were actively supplanted from human life by chemical products. However, despite the enormous progress in this area, in recent decades, the problems of serious side effects of synthetic chemicals on the environment have become more and more pronounced. For example, prolonged use of pesticides in agriculture has caused serious harm to nature and human health (Kumar and Kumar, 2019).

In the field of pharmacology, plants have been the basis for the creation of highly effective medicinal drugs and dietary additives that are used to correct human health. In the field of agriculture, insecticides of new generations, defoliants, attractants, many of which are of plant origin, have been obtained. Active substances from plants are necessary for various fields of industry, as well as promising for use in biotechnology (Fakheri *et al*., 2011).

The growing amount of information on the diversity of biological activities in plants requires not only an assessment of the role of metabolites in their life and protection against biotic and abiotic stresses but also encourages a comprehensive study of the chemical composition of wild plant species, depending on their habitats. To replenish the assortment of plants, the rigorous and comprehensive studies of the chemical composition and biological properties of individual compounds and phytocomplexes are necessary. The theoretical aspects of the study of secondary plant products as growth regulators, gene expression modulators, and signal transmitters are of great interest. The study of this problem is promising from the point of view of evolutionary transformations of the molecules of secondary compounds and, possibly, the mechanisms of their action on various organisms. According to WHO, the pharmaceutical market in some countries is already 80% represented by plant preparations. Moreover, the production of herbal preparations in the world reaches 60 billion US dollars (WHO traditional…, 2002; Willcox and Bodeker, 2004). Currently, plants containing

flavonoids (biologically active compounds belonging to the class of plant polyphenols) are considered to be the most promising as a source of drug raw materials. Due to the wide variety of their structures and the wide distribution in various plants, flavonoids, and plants containing them are increasingly being studied as a source of phytopreparations in pharmacy (Harborne and Mabry, 1982; Wagner, 1993; Kurkin, 2009; Makarova and Makarov, 2010; Kurkina, 2012; Abdrakhmanova *et al*., 2019).

Flavonoids belong to natural compounds and are part of many phytopreparations that exhibit specific biological activity of a targeted action (Abhay, 2013; Karak, 2019). Flavonoids in the human body act both on enzyme systems and immune and metabolic processes, causing various effects positive for the body (Hoensch and Oertel, 2015; Panche *et al*., 2016). Many scientists argue that the diversity of the biological effects of flavonoids is due to their antioxidant activity (Pandey *et al*., 2010; Kurkin *et al*., 2013; Mishra *et al*., 2013). Flavonoids also have antimicrobial, anti-inflammatory, wound healing, vessel-strengthening, antitumoral, antiulcerogenic, and other actions. Flavonoids can be a free radicals scavenger and inhibit lipid peroxidation (Gerdin and Srensso, 1983; Cowan, 1999; Cushnie and Lamb, 2005; Nagendra Prasad *et al*., 2010; Zandi *et al*., 2011). Flavonoids as antioxidants play an important role in preventing violations of the structure and functions of the liver in various pathologies, accelerating the regeneration and restoring the functional activity of hepatocytes, especially in the treatment of acute and chronic hepatitis and cirrhosis (Chen *et al*., 2014; Sánchez-Salgado *et al*., 2019). The most active were flavonols quercetin and myricetin, which showed high antiviral activity (Kaul *et al*., 1985; Wu *et al*., 2008). Currently, flavonoid preparations in the form of various dosage forms are widely used in medical practice: tablets, ointments, tinctures, extracts, powders, dragees, and capsules (Pashtetskiy *et al*., 2020).

Modern wound coverings include films. The wound covering, as a dressing, must meet the following requirements: create an optimal microenvironment for wound healing, prevent the penetration of microorganisms into the wound, have elasticity, the ability to model surfaces with complex relief, without pyrogenic, antigenic and toxic effects, local irritating and allergic actions. Also, for artificial wound coverings, the following properties are highly desirable: transparency, the ability to observe a wound, the ability to be a drugs vehicle (antibacterial, reparants), ease of use of films for medical personnel, easy removal of the film from the surface of the skin.

Kazakhstani scientists are also intensively searching for new effective, environmentally friendly medicines based on herbal raw materials. Northern Kazakhstan is the richest region of the medicinal flora habitat, which is represented by *Linosyris villosa*. The chemicals included in the medicinal plant *Linosyris villosa* are currently equally interesting as objects of study in phytochemistry, pharmacy, and medicine (Nazarova *et al*., 2013; Ramilyeva *et al*., 2019).

The object of study: *Linosyris villosa*, collected in the flowering phase in Northern Kazakhstan (Korulkin *et al*., 2007). This is a perennial herbage plant with numerous stems and a horizontal rhizome 30-40 cm high. The leaves are oblong-linear, sessile, whole-cut. The flowers are collected in a cymose inflorescence of yellow color. The plant blooms in late July and early August. *Linosyris villosa* grows in the European part of Russia, Western Siberia, the Caucasus, Ukraine, and Kazakhstan.

The chemical composition of the plant has not been studied. In folk medicine, *Linosyris villosa* is used in the treatment of bronchial asthma, angina pectoris, toothache, and rheumatic pains (Kyusev, 2002). The following dosage forms were obtained based on *Linosyris villosa*: ointment, tinctures, adhesive plasters, which have antidermatitis, anti-inflammatory, and antiseptic effects, so further study of the plant is of extreme interest (Nazarova and Bakumova, 2015; Nevkrytaya *et al*., 2020).

The problem of the article is the search and creation of drugs based on the flora of Northern Kazakhstan. The scientific novelty lies in the fact that the chemical composition and biological activity of the *Linosyris villosa*, growing in the territory of Northern Kazakhstan, is being studied for the first time. The practical significance of the work is defined as follows: biologically active compounds from the *Linosyris villosa* plant were obtained for the purpose of studying and applying them in medicine and agriculture.

The purpose of the article is to study the chemical composition of the *Linosyris villosa* by physicochemical methods of analysis, as well as the creation of dosage forms based on it.

2. MATERIALS AND METHODS

The extraction of biologically active substances from the plant was carried out in a

Soxhlet's apparatus using various solvents (96% aqueous solution, 70% aqueous solution). Extraction was carried out at a temperature of 40 ℃ for 7 days. Aluminium oxide and nylon as adsorbents were pre-established and used for the separation of flavonoids contained in extracts of the *Linosyris villosa* plant.

Determination of pesticides in alcohol fractions of *Linosyris villosa*. The ECD_pesticides (HOP).M method was calibrated to detect organochlorine compounds for the following pesticides: hexachlorocyclohexane (HCH) and its isomers: alpha-HCH, beta-HCH, gamma-HCH; 4,4-dichlorodiphenyltrichloroethane (DDT) and its metabolites: 4,4-dichlorodiphenyldichloroethylene (DDE), 4,4-dichlorodiphenyldichloroethane (DDD). The calibration of the pesticide mixture was carried out at 3 levels. Calibration solutions were prepared from standard pesticide samples (pesticide solutions in hexane with a concentration of 100 µg/ml), from which calibration solutions with concentrations of 16.667 µg/ml (level 3), 8.333 µg/ml (level 2) were prepared by sequential dilution 4.167 mcg/ml (level 1). To carry out the calibration, $5 \cdot 10^{-3}$ cm^3 of the solution was sequentially introduced into the chromatograph evaporator using a microsyringe for each calibration concentration. Each solution was chromatographed twice, calculating the average peak area value of the determined organochlorine pesticide in the chromatogram. The correlation coefficients for the calibration lines for all three concentrations (3 levels) were: 0.99986 for α-HCH, 0.99575 for β-HCH, 0.99942 for γ-HCH, 0.99912 for DDE, 0.99969 for DDD.

The studies were carried out on an Agilent Technologies 7890B GC System gas chromatograph with an electron capture detector (ECD) and a nitrogen phosphorus detector (NPD), on an HP-5 capillary column.

The concentration of residual amounts of organochlorine pesticides, mg/kg, in the analyzed sample, was calculated following the calibration curves. The arithmetic mean value of the results of three parallel determinations was taken as the final analysis result. The discrepancy between the parallel determinations did not exceed the repeatability limit. The repeatability limit for all determining organochlorine pesticides is 20%. The results were considered reliable if the discrepancy between parallel determinations did not exceed the repeatability limit. The repeatability limit for all detectable organochlorine pesticides on a gas chromatograph with an electron-capture and nitrogen-phosphorus detector is 20% according to the determination method of ST RK 2011-2010 "Water, food, feed and tobacco products. Determination of organochlorine pesticides by chromatographic methods".

The toxicity of polymer films was determined using an AT-05 image toxicity analyzer. This indicator is an alternative to the "local skin irritant action" indicator and does not require the use of experimental animals during testing (Kurkin *et al*., 2013).

The separation of the substances from the aqueous-alcoholic extract was made using the method of column adsorption chromatography. Aluminum oxide, nylon, and polisorb were used as adsorbents. The best separation results were achieved on nylon and aluminum oxide. On nylon, carbohydrates were separated as related substances, aglycones and glycosides of flavonoid nature were separated.

Aglycones were separated on an aluminum oxide column, in particular, myricetin-containing fractions were obtained. To identify the aglycon, myricetin, it was accumulated, dissolved in 96% alcohol, and rechromatographed on an aluminum oxide column. The resulting eluate was evaporated to dryness and crystallized many times from 80% ethanol in the presence of activated charcoal during which received yellowish crystals with a melting point of 318-320°C. The substance was investigated by two-dimensional paper chromatography in I R_f = 0.50 and II R_f = 0.00 systems (Figure 1).

During the study, 4 options for films that differ in composition and production technology were obtained.

Option 1. A portion of polyvinyl alcohol (PVA) weighing 1 g was poured into 40 ml of distilled water and left to swell for 24 hours. Then the PVA solution was heated on a tile with an asbestos mesh for 30 minutes. Subsequently, 1 ml of glycerol was added and heated for another 10 minutes. The resulting solution was poured onto a fat extracted base glass lubricated with glycerol. The film dried in 24 hours. The film was thin, transparent, and elastic. The main disadvantage of the film obtained is that it was not well fixed on the skin.

Option 2. A portion of PVA weighing 1.25 g was poured into 40 ml of distilled water and left for 24 hours to swell. Then the PVA solution was heated on the tile for 30 minutes. Subsequently, 1 ml of glycerol was added and heated for another 10 minutes. To the obtained carrier, when

heating, 1 ml of alcohol was poured and left to dry. Such a film dried longer than a film obtained from a carrier.

Option 3. To the carrier of the film obtained in option 2 was added 1 ml of water while heating. The resulting carrier was poured onto a base glass for drying. The film did not dry for a week. The resulting films did not meet the requirements since their drying time increased in several times.

Option 4. The carrier was prepared according to option 1, then, when heated, 2 ml of glycerol was added to the prepared carrier. The resulting solution was poured onto fat extracted base glass, lubricated with glycerol. The film dried in 24 hours. Such a film was thin, transparent, elastic, easily fixed on the skin and did not deform when moving.

Then, the active substance (*Linosyris villosa* extract) was added dropwise to the resulting solution while stirring. The resulting solution was poured onto a fat extracted base glass, lubricated with glycerol. The film dried in 24 hours. The addition of the active substance did not change the properties of the film. The substance was distributed evenly over the entire area of the film. It was concluded that the addition of alcohol and water to the film carrier increased the drying time of the film. Such films do not meet the requirements. The film carrier, with the addition of glycerin and the active substance, meets all the requirements for medicated films and has a prolonged effect (Smiyan *et al*., 2018).

The test of the obtained films was carried out in an experiment on the skin of patients of the surgical department, after surgical interventions, by such indicators as drying time, film quality, and fixation on the skin (Table 1).

To verify the safety of the obtained medicated film, four aqueous extracts from a polymer film were prepared according to option No. 4, 2 aqueous extracts without an active substance and 2 aqueous extracts with the addition of an active substance with a different concentration, one for each concentration of the active substance (*Linosyris villosa* extract). Previously, it was necessary to evaluate the toxicity of the polymeric material (PVA) used to prepare the films. According to the methodology, an extract from the granules of the polymer material is prepared as follows: the M/V ratio of the PVA granules to distilled water should be 0.1 g per 1 ml of water. For testing, 100 ml of extract was prepared. 10 g of PVA was poured into 150 ml of water and kept in a thermostat at a temperature of 70 ℃ for 24 hours. After that, the extract was passed through a paper filter to relieve the swollen PVA. In parallel, the same volume of distilled water was thermostated under the same conditions to obtain a control sample (Ќ).

The obtained extracts were kept in a thermostat at a temperature of 70 ℃ for 24 hours. In parallel, the same volume of distilled water was thermostated under the same conditions to obtain a control sample (Ќ). The degree of toxicity was determined by comparing the value of the selected test parameter of the test sample (solution) and the value of the same test parameter of the control sample (solution). When determining the toxicity index on the AT-05 image toxicity analyzer, the time-weighted average of the advancement of the sperm suspension was chosen as a test parameter. For the experiment, it was prepared the control and experimental solutions. The experimental solution is prepared from the calculation: 10 ml of aqueous extract, 0.4 g of glucose, 0.1 g of sodium citrate. The control solution is prepared similarly to the experimental one, but instead of the aqueous extract of the polymer film it includes 10 ml of distilled water. After preparation, the control and experimental solutions using a 0.4 ml dispenser were placed in thin section test tubes. The tubes are closed with stoppers and installed in the sample preparation unit (SPU).

The preparation of a uterine sperm suspension was carried out as follows: 1.5 ml of a control solution (solution for sperm thawing) was placed in a test tube using a dispenser. A tube with a solution for thawing is installed in the SPU and warmed up to 40 ℃ for at least 5 minutes. Then, we removed "straw" with sperm from the Dewar flask using surgical forceps, the end of which is cooled in liquid nitrogen. Holding the straw in the middle with forceps, we cut the polymer packaging on both sides with scissors and place the straw in a test tube. After 10-15 minutes, when the contents of the “straw” completely goes into solution, the tube was shaken several times and returned to its place for further heating. The contents of the tube were warmed for 5-7 minutes and periodically shaken. After that, 0.1 ml of the uterine sperm suspension was added to the tubes with control and experimental solutions using a dispenser. The contents of each tube were mixed. After thorough mixing, the capillaries of the device were filled with work solutions.

The capillaries were filled as follows: the capillary was taken in the right hand so as not to touch the central part of the working surface of the

capillary. The forefinger of the right-hand clamps the end of the capillary and dip the opposite end into a test tube with a work solution with cells to a depth of at least 5 mm. Then the clamped end of the capillary was released. Due to the capillary effect, the solution fills the capillary. The filling process is monitored visually. The capillary is removed from the tube, the wet end is wiped with dry gauze, and the capillary is placed in the carriage at the appropriate position. The carriage is closed with a lid and placed in the analyzer block. 5 capillaries filled with a control sample and 5 capillaries of a test sample were placed in the analyzer unit. 5 cycles of measurements were carried out. The toxicity index was calculated according to the following formula (Equation 1), where I_t is the toxicity index, t_{tw}^{o} t_{tw}^{k}, are the time-weighted averages of the mobility time of the sperm suspension in experimental and control capillaries with solutions, respectively.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

3. RESULTS AND DISCUSSION:

3.1. Obtaining myricetin fractions from the *Linosyris villosa* plant

The object of study was the *Linosyris villosa* plant, the aerial part of which was collected in Northern Kazakhstan during the flowering phase. The moisture content of the raw materials is 8.10%, the ash content is 4.45%. The number of extractives, depending on the type of solvent, is presented in Table 2. Subsequently, to obtain extractives, 70% ethanol was used as the best extraction solvent for the *Linosyris villosa* plant, according to the results of Table 2. In order to establish the composition of the aqueous-alcoholic extract, a qualitative analysis was carried out using specific reagents (Table 3).

Analyzing the results presented in Table 3, it should be concluded that flavonoids are contained in all extracts obtained from the *Linosyris villosa* plant. In the interaction of ammonia vapors with the extract, a yellow color develops, indicating the presence of flavonoids in the extract, the molecule of which contains a carbonyl group that reacts with ammonia to form yellow imines. When the extract interacts with an alcoholic solution of aluminum chloride, yellow color forms, indicating the presence of flavonoids containing hydroxyl groups in 3 and 5 positions, due to which complex compounds colored in yellow forms. As a result of the interaction of a 1% aqueous solution of lead acetate with the extract, an orange-yellow precipitate forms, indicating the presence of flavones and flavanols in the aqueous-alcoholic extract. During the interaction of a 1% alcohol solution of ninhydrin with the extract, a blue-violet color develops when heated, due to the formation of a blue-violet Ruehmann purpura, indicating the presence of amino acids in the extract. During the interaction of a 1% solution of ferrous ammonium sulfates (FAS) with the extract, green color was observed, and when left to stand, a dark green precipitate formed, indicating the presence of hydrolyzable and condensed tannins in the extract.

Thus, on the basis of qualitative analysis, it can be assumed that the following classes of compounds are present in the extract: flavonoids (flavones, flavonols), tannins, amino acids, carbohydrates, and other compounds. The aqueous-alcoholic extract was also studied by two-dimensional paper chromatography (PC) in systems B:A:W (butanol-acetic acid-water) in the ratio (4:1:5) (I) and 2% acetic acid (II). According to OFS.1.2.1.2.0002.15 “Chromatography on paper”, two-dimensional paper chromatography allows us to establish the authenticity, purity, and quantification of the substance sampled involves the sequential passage of the released substances on a sheet of filter paper when moving the mobile phase in two perpendicular directions along the capillaries of the paper, which allows for a clearer separation of the mixture of analytes. Five substances related to flavonoids (aglycones, glycosides), phenolic acids, as well as carbohydrates, amino acids, and traces of tannins were found on the chromatogram.

For aglycon, the IR spectrum was taken in Kbr tablets. In the IR spectrum there were absorption bands in the range of 1650 cm^{-1} (C=O); 3450 cm^{-1} (-OH); in the region of in the range of 2850 cm^{-1}, 2940 cm^{-1} (C-H) of the E-ring; in the range of 1480, 1520, 1610 cm^{-1} (C=C) of the E-ring. Thus, on the basis of identification tests, melting point, comparison with myricetin marker, literature data, and IR spectroscopy, the obtained aglycon was identified as myricetin (Figure 2).

3.2. Determination of the toxicity of myricetin fractions produced from the *Lynosyris villosa* on the content of pesticides

To study myricetin fractions, pesticides were selected, which occupy one of the leading positions in terms of hazard and prevalence in the environment. The danger of HCH and its isomers, as well as DDT and its metabolites, is that they are able to persist and accumulate in environmental objects for a long time. And despite the fact that they have not been used for a long time in Kazakhstan, however, they can still be found in environmental objects due to their high persistency.

Determination of pesticides in alcohol fractions of *Linosyris villosa*. The ECD_pesticides (HOP).M method was calibrated to detect organochlorine compounds for the following pesticides: hexachlorocyclohexane (HCH) and its isomers: alpha-HCH, beta-HCH, gamma-HCH; 4,4-dichlorodiphenyltrichloroethane (DDT) and its metabolites: 4,4-dichlorodiphenyldichloroe_ thylene (DDE), 4,4-dichlorodiphenyldichloro_ ethane (DDD). The studies were carried out on an Agilent Technologies 7890B GC System gas chromatograph with an electron capture detector (ECD) and a nitrogen phosphorus detector (NPD), on an HP-5 capillary column.

According to the results of three parallel studies of myricetin fractions, the organochlorine compounds were not found in the samples; there were no peaks on the chromatogram corresponding to the determined pesticides (Figure 3).

3.3. Study of the safety parameters of medicated films based on the *Linosyris villosa* plant

The specified interval of the toxicity index is $70 < I_t < 120$. Belonging to this specified interval allows the tested products to be considered non-toxic. The boundaries of the interval are established on the basis of the analysis of the results of parallel tests on animals and on the semen of a bull, which made it possible to find a range where the toxic effect is not found in animal tests. The results of the analysis of the PVA toxicity are presented in Table 4.

Calculation of the toxicity index for PVA polymer material based on the results of 5 cycles (Equation 2). Since the value is 70 <84.3 <120, therefore, the polymer material can be considered safe for use and the manufacture of polymer films. Next, the film was prepared according to the recipe of experiment No. 4. We prepared a polymer film and an extract from it: Sample No. 1 (O_1) is the polymer film without adding an active substance; Sample No. 2 (O_2) is the polymer film with an active substance; Sample No. 3 (O_3) is the polymer film with an active substance, the dose of which is doubled.

An extract for dressings in contact with human skin is prepared as follows: S (area) of the test film is taken to V (volume) of distilled water in ratio 1/1 cm²/ml. Moreover, both surfaces of the polymer film are taken into account. To obtain the desired volume, we took 3 samples of a film measuring 10 x 10 cm and filled in a beaker with 200 ml of distilled water. A beaker was covered with a watch glass, and extraction was carried out at a temperature of 40 ℃ for 7 days. In parallel, the same volume of distilled water was thermostated under the same conditions to obtain a control sample. Samples were taken, and the toxicity index was measured on the 1st, 3rd, and 7th days of extraction. The results of the experiment on the toxicity of polymer extracts after extraction on day 1 are presented in Table 5.

Calculation of the toxicity index for 3 samples according to the results of 5 measurements: $I_t\,(O_1) = \frac{185}{226} \times 100\% = 81.9\%$, $I_t\,(O_2) = \frac{177}{226} \times 100\% = 78.3\%$, $I_t\,(O_3) = \frac{177}{226} \times 100\% = 78.3\%$. The results of toxicity after extraction on the 3rd day are presented in Table 6.

Calculation of the toxicity index for 3 samples according to the results of 5 measurements, after 3 days of extraction: $I_t\,(O_1) = \frac{255}{315} \times 100\% = 80.9\%$, $I_t\,(O_2) = \frac{251}{315} \times 100\% = 79.6\%$, $I_t\,(O_2) = \frac{251}{315} \times 100\% = 79.6\%$. The results of toxicity after extraction of polymer films extracts for 7 days are presented in Table 7.

The toxicity index was calculated for 3 samples according to the results of 5 measurements, after 7 days of extraction: $I_t\,(O_1) = \frac{271}{348} \times 100\% = 77.9\%$, $I_t\,(O_1) = \frac{271}{348} \times 100\% = 77.9\%$, $I_t\,(O_1) = \frac{271}{348} \times 100\% = 77.9\%$. The results showed that PVA-based polymer films with and without the addition of the *Linosyris villosa* extract do not exhibit toxic effects.

3.4. Determination of the skin irritant action of medicated films based on the *Linosyris villosa* plant

The skin irritant action of the films was tested in parallel on 3 samples:

– sample No. 1 (O_1) is the polymer film

without adding an active substance;

– sample No. 2(O_2) is the polymer film with an active substance;

– sample No. 3 (O_3) is the polymer film with an active substance, the dose of which is doubled.

The action was studied on white mice by immersion of the tails in the extract. The result was compared with a control sample (distilled water). To obtain 1200 ml of the extract, a total of 600 cm² of each sample was taken, the film was placed in a beaker covered with a porcelain dish, extraction was carried out at a temperature of 40 ℃ for ten days. In parallel, the same volume of distilled water was thermostatically controlled under the same conditions to obtain a control sample. Within ten days, samples were taken, and studies were conducted.

White mice were fixed in special cages so that their tails were immersed 2/3 in the solution. The exposure was carried out for 2 hours. The reaction was taken into account immediately after the end of the exposure by the presence of local skin changes (Tables 8-10).

The data showed that the polymer film does not have a skin irritant action and is safe to use.

3.5. Study of the drug "VITIN" regarding antianemic activity

The drug "Vitin" obtained by aqueous-alcoholic extraction of the *Linosyris villosa* plant. The following classes of natural compounds were found in the preparation by phytochemical analysis using specific reagents: flavonoids, essential oils, carbohydrates, amino acids, phenolic acids, vitamins, trace elements, and tannins in the form of traces. The drug was studied in the toxicology laboratory "Deri-Dermek" for toxicity, irritating, healing effects, and blood formation. Analysis of the data obtained indicates that Vitin is non-toxic, stimulates the healing process, has an antiseptic effect, and normalizes blood formation.

The effect of the Vitin drug on blood formation was investigated on the basis of a regional hospital on patients in the hematological department during inpatient treatment and during outpatient treatment after the discharge of patients from the hospital. More than 30 patients with anemia and diseases of the internal organs were examined (Table 11).

Studies were performed on 30 patients (22 women and 8 men) aged 17 to 70 years (mean age 40 ± 3.5 years) (Table 12). The patients were divided into 3 groups. Patients of the 1st group took Vitin as a monotherapy, patients of the 2nd group took Vitin in combination therapy with iron preparations. The control group 3 consisted of patients taking iron preparations without Vitin.

Evaluation of the drug effectiveness was carried out after a 2-3 week course of treatment according to the indicators of a complete blood count for two months. Prior to inclusion in the study protocol, at the entrance to its completion, and at the end of the study, all patients underwent a complete blood count to calculate the number of red blood cells and hemoglobin, color index, white blood cell count, and WBC differential. The treatment was stopped when achieving normal blood counts and performance status.

4. CONCLUSIONS:

The object of the study was the *Linosyris villosa* plant, collected in the flowering phase in Northern Kazakhstan. To establish the quality of raw materials, pharmacopoeial indicators were determined: humidity (8.10%), ash content (4.45%), and the amount of extractives (16.88%). The optimal flavonoid extractant for the *Linosyris villosa* was experimentally established, which is the aqueous-alcoholic solution (70% ethanol). Using sequential extraction methods and two-dimensional paper chromatography in systems-I (butanol-acetic acid-water) (B:A:W) in a ratio of 4:1:5 and system II (2% acetic acid) the presence of flavonoids in all extracts was observed. Using adsorption chromatography on aluminum oxide, and then distribution chromatography on nylon, a flavonoid aglycone was isolated, the structure of which was proved by physicochemical methods of analysis. Based on qualitative reactions, melting point, comparison with a marker, literature data, and IR spectroscopy, the isolated substance was identified as a flavonoid-based aglycone – myricetin.

The aqueous-alcoholic extract from the *Linosyris villosa* plant was studied for safety by the residual amount of organochlorine pesticides in the extract. There were no pesticides in the studied extract; a conclusion was drawn about the possibility of using plant materials based on *Linosyris villosa* for the manufacture of dosage forms. Polyvinyl alcohol-based medicated films were obtained with the addition of an active substance based on myricetin containing extracts from the *Linosyris villosa* plant. A study of the obtained medicated films for the presence of toxic

and skin irritant actions were conducted. The results showed that polyvinyl alcohol-based polymer films with and without the addition of *Linosyris villosa* extract did not exhibit toxic effects. The reaction to skin irritant action was zero points (reaction – negative).

The aqueous-alcoholic extract of the *Linosyris villosa* plant was investigated for the presence of antianemic activity. According to the results of the analysis, in 13% of patients the treatment effect is insignificant, and in 87% of patients there was an improvement in blood values (increase in hemoglobin, an increase in the number of red blood cells, almost all patients returned normal color index), the number of white blood cells increased to normal, and the WBC differential improved. The conclusion was made that the aqueous-alcoholic extract of the *Linosyris villosa* plant is an effective drug for the treatment of patients with anemia of various severity.

5. REFERENCES:

1. Abdrakhmanova, S. A., Zhanzakova, Z.Z., Turganbekova, A. A., *and* Saduakas, Z.K. (2019). Assessment of hematopoetic stem cell molecular engraftment based on STR analysis. *Cellular Therapy and Transplantation, 8*(3), 26-27.
2. Abhay, K. (2013). Pandey chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, *2013*, article number 162750. https://doi.org/10.1155/2013/162750
3. Chen, X., Mukwaya, E., Wong, M.-S., *and* Zhang, Y. (2014). A systematic review on biological activities of prenylated flavonoids. *Pharmaceutical Biology, 52*, 655–660. https://doi.org/10.3109/13880209.2013.853809.
4. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564-582.
5. Cushnie, T.P.T., *and* Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343-356.
6. Fakheri, B., Rahimi, M., Tavassoli, A., *and* Raissi, A. (2011). Application of biotechnology in production of medicinal plants. *American-Eurasian Journal of Agricultural and Environmental Science*, 11. Retrieved from https://www.researchgate.net/publication/266231628_Application_of_Biotechnology_in_Production_of_Medicinal_Plants.
7. Gerdin, B., *and* Srensso, E. (1983). Inhibitory effect of the flavonoid on increased microvascular permeability induced by various agents in rat skin. *International Journal of Microcirculation, Clinical, and Experimental*, *2*(1), 39-46.
8. Harborne, J.B., *and* Mabry, T.J. (Eds.). (1982). *The flavonoids: Advances in research.* London, United Kingdom; New York City, New York: Chapman and Hall.
9. Hoensch, H.P., *and* Oertel, R. (2015). The value of flavonoids for the human nutrition: Short review and perspectives. *Clinical Nutrition Experimental*, *3*, 8-14. https://doi.org/10.1016/j.yclnex.2015.09.001
10. Karak, P. (2019). Biological activities of flavonoids: an overview. *International Journal of Pharmaceutical Sciences and Research*, *10*(4), 1567-74. DOI: 10.13040/IJPSR.0975-8232. 10(4).1567-74.
11. Kaul, T., Middleton, E., *and* Ogra, P. (1985). Antiviral effect of flavonoids on human viruses. *Journal of Medical Virology*, *15*, 71–79.
12. Korulkin, D. Yu., Abilov, J. A., Muzychkina, R.A., *and* Tolstikov, G.A. (2007). *Natural flavonoids*. Novosibirsk, Russian Federation: GEO.
13. Kumar, V., *and* Kumar, P. (2019). Pesticides in agriculture and environment: Impacts on human health. In *Contaminants in agriculture and environment: health risks and remediation, chapter: Chapter 7* (pp. 76-95). Haridwar, India: Agro Environ Media.
14. Kurkin, V. A. (2009). *Fundamentals of herbal medicine: A manual for students of pharmaceutical universities*. Samara, Russian Federation: Etching LLC, GOU VPO SamGMU of Roszdrav.
15. Kurkin, V. A., Kurkina, A.V., *and* Avdeeva, E.V. (2013). Flavonoids as biologically active compounds of medicinal plants. *Fundamental Research*, *11-9*, 1897-1901.
16. Kurkina, A.V. (2012). *Flavonoids of pharmacopeia plants*. Samara, Russian Federation: LLC “Etching:”; SBEI HPE SamSMU of the Ministry of Health and Social Development of Russia.
17. Kyusev, P.A. (2002). *A complete directory of medicinal plants*. Moscow, Russian

Federation: Eksmo.

18. Makarova, M.N., *and* Makarov, V.G. (2010). *Molecular biology of flavonoids (chemistry, biochemistry, pharmacology): a guide for doctors*. St. Petersburg, Russian Federation: Lema.
19. Mishra, A., Sharma, A.K., Kumar, S., Saxena, A.K., *and* Pandey, A.K. (2013). Bauhinia variegata leaf extracts exhibit considerable antibacterial, antioxidant and anticancer activities. *BioMed Research International*, *2013*, article number 915436.
20. Nagendra Prasad, K., Xie, H., *and* Hao, J. (2010). Antioxidant and anticancer activities of 8-hydroxypsoralen isolated from wampee [Clausena lansium (Lour.) Skeels] peel. *Food Chemistry*, *118*(1), 62-66.
21. Nazarova, V. D., *and* Bakumova, E. V. (2015). Flavonoids of the *Linosyris villosa* plant. *Chemical Journal of Kazakhstan*, *2*, 348-353.
22. Nazarova, V. D., Bektemisova, A. U., *and* Bakumova, E. V. (2013). The study of polyphenols in the plant *Linosyris villosa*. *Materials of the Scientific and Practical Conference "Kozybaev Readings – 2013: Kazakhstan in the World Cultural and Civilization Processes*, *III*, 194-198.
23. Nevkrytaya, N. V., Pashtetskiy, V. S., Novikov, I. A., Korotkikh, I. N., *and* Tkhaganov, R. R. (2020). Variability of component composition of melissa officinalis l. essential oil depending on the climatic conditions of the region of cultivation. *Khimiya Rastitel'nogo Syr'ya, 1*, 257-263.
24. Panche, A. N., Diwan, A. D., *and* Chandra, S.R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, article number e47. DOI:10.1017/jns.2016.41.
25. Pandey, A. K., Mishra, A. K., Mishra, A., Kumar, S., *and* Chandra, A. (2010). Therapeutic potential of zeylanicum extracts: an antifungal and antioxidant perspective. *International Journal of Biological and Medical Research*, *1*, 228-233.
26. Pashtetskiy, V. S., Turin, E. N., Izotov, A. M., Abdurashytov, S. F., Gongalo, A. A., *and* Zhenchenko, K. G. (2020). Effect of Pisum sativum L. seed treatment with the complex of microbiological preparation on the plants' growth and development under direct sowing. *IOP Conference Series: Earth and Environmental Science, 422*(1), article number 012012.
27. Ramilyeva, I. R., Burkitbaev, Zh. K., Abdrakhmanova, S. A., Turganbekova, A. A., Baimukasheva, D. K., *and* Zhiburt, E. B. (2019). Distribution pattern for HLA specificities in the patients with acute myeloid leukemia. *Medical Immunology (Russia), 21*(5), 965-972.
28. Sánchez-Salgado, J. C., Estrada-Soto, S., *and* García-Jiménez, S. (2019). Analysis of flavonoids bioactivity for cholestatic liver disease: Systematic literature search and experimental approaches. *Biomolecules*, *9*(3), article number 102. https://doi.org/10.3390/biom9030102
29. Smiyan, O. I., Loboda, A.M., Manko, Y.A., Bynda, T. P., Popov, S.V., Petrashenko, V.O., Sichnenko, P.I., Romaniuk, O.K., Gorbas, V.A., Zagorodnii, M.P., Smiian-Horbunova, K.O., Kosarchuk, V.V., Ovsianko, O.L., *and* Kolesnikova, M.V. (2018). Dynamics of content of some minerals in teenagers with cardiovascular system pathology against the background of chronic tonsillitis. *Wiadomosci lekarskie (Warsaw, Poland: 1960), 71*(4), 861-864.
30. Wagner, H. (1993). *Pharmazeutische biologie. Drogen und ihre Inhaltsstoffe*. Stuttgart, Germany: Gustav Fischer Verlag.
31. WHO traditional medicine strategy 2002–2005 Geneva: WHO. (2002). Retrieved from https://www.who.int/medicines/publications/traditionalpolicy/en/.
32. Willcox, M. L., *and* Bodeker, G. (2004). Traditional herbal medicines for malaria. *British Medical Journal*, *329*, 1156-1159.
33. Wu, D., Kong, Y., *and* Han, C. (2008). d-Alanine: d-alanine ligase as a new target for the flavonoids quercetin and apigenin. *International Journal of Antimicrobial Agents*, *32*, 421-426.
34. Zandi, K., Teoh, B. T., Sam, S. S., Wong, P. F., Mustafa, M. R., *and* Abubakar, S. (2011). Antiviral activity of four types of bioflavonoid against dengue virus type-2. *Virology Journal*, *8*, article number 560.

$$I_t = \frac{t^o_{tw}}{t^k_{tw}} \times 100\% \quad \text{(Eq. 1)}$$

$$I_t = \frac{145}{172} \times 100\% = 84.3\%. \quad \text{(Eq. 2)}$$

Table 1. *medicinal films test results*

Film variant	Drying time	Organoleptic characteristics of films	Fixation on the skin
1	24 hours	thin, transparent, elastic	short-term fixation (for 12 hours), deformation during movement
2	4 days	absence of elasticity	absence of fixation
3	6 days	absence of elasticity	absence of fixation
4	24 hours	thin, transparent, elastic	long-term fixation (for 24 hours), abscense of deformation during movement

Table 2. *Determination of the amount of extractives*

Raw material	The number of extractives, %, in solvents:		
	hexane	ethanol (96% aqueous solution)	ethanol (70% aqueous solution)
Aerial part of *Linosyris villosa*	3.79	10.10	16.88

Table 3. *Qualitative study of the extract*

Extract	Reagents				
	NH_3 (fume)	$AlCl_3$ 1% alcohol solution	Pb $(CH_3COO)_2$ 1% aqueous solution	Ninhydrin 1% alcohol solution	Ferrous ammonium sulfate 1% aqueous solution
70% ethanol	bright yellow	yellow	orange-yellow	violet	green

Table 4. *Time-weighted average values of sperm suspension motility time*

No. of capillary	K1	K2	K3	K4	K5	t^k_{tw}	K6	K7	K8	K9	K10	t^o_{tw}
Cycle No.												
1	174	195	212	117	176	175	218	147	130	129	116	148
2	161	207	186	163	179	179	226	151	232	144	108	151
3	152	186	236	115	210	180	220	147	196	145	96	153
4	140	175	184	192	156	170	200	104	156	120	118	140
5	148	163	180	136	160	157	200	159	190	126	103	135
Average value						172						145

Table 5. *Weighted average values of the mobility time of the sperm suspension for polymer extracts after extraction in 1 day*

No. of capillary	**K1**	**K2**	**K3**	**K4**	**K5**	t_{tw}^{k}	**K6**	**K7**	**K8**	**K9**	**K10**	t_{tw}^{o}**1**
Cycle No.												
1	253	296	234	198	156	227	186	123	271	220	154	191
2	263	231	284	202	174	231	202	105	197	236	186	185
3	198	168	303	241	196	221	174	134	200	208	174	178
4	185	224	268	271	165	223	210	132	203	215	186	189
5	307	185	242	237	178	230	187	135	206	198	181	181
Average value						226						
No. of capillary	**K11**	**K12**	**K13**	**K14**	**K15**	t_{tw}^{o}**2**	**K16**	**K17**	**K18**	**K19**	**K20**	
1	164	157	183	170	200	175	146	168	174	179	211	176
2	210	135	157	203	189	179	154	156	157	201	214	176
3	185	134	210	200	174	181	157	154	213	213	163	180
4	206	148	194	165	176	178	165	148	181	183	176	171
5	178	153	176	169	181	171	170	167	219	169	181	181
Average value						177						177

Table 6. Weighted average values of the mobility time of the sperm suspension for polymer extracts after extraction in the 3rd day

No. of capillary	**K1**	**K2**	**K3**	**K4**	**K5**	t_{tw}^{k}	**K6**	**K7**	**K8**	**K9**	**K10**	t_{tw}^{o}**1**
Cycle No.	356	296	316	310	300	316	256	263	246	287	268	264
1	302	316	299	326	325	314	254	234	248	254	277	253
2	297	265	326	341	350	316	210	218	295	235	274	246
3	307	325	330	289	325	315	269	257	231	245	278	256
4	307	316	296	310	348	315	260	274	236	253	262	257
5	356	296	316	310	300	316	256	263	246	287	268	264
Average value						315						255
No. of capillary	**K11**	**K12**	**K13**	**K14**	**K15**	t_{tw}^{o}**2**	**K16**	**K17**	**K18**	**K19**	**K20**	t_{tw}^{o}**3**
Cycle No.												
1	226	234	248	248	263	244	215	265	237	242	259	244
2	235	248	265	235	247	246	234	219	268	237	248	241
3	258	267	231	255	249	252	284	236	247	259	235	252
4	273	245	261	268	254	260	249	235	268	245	247	249
5	264	239	243	248	275	254	243	235	236	257	249	244
Average value						251						246

Table 7. *Weighted average values of the mobility time of the sperm suspension for polymer extracts after extraction at 7 days*

No. of capillary	**K₁**	**K₂**	**K₃**	**K₄**	**K₅**	t_{tw}^{k}	**K₆**	**K₇**	**K₈**	**K₉**	**K₁₀**	t_{tw}^{o}**1**
Cycle No.												
1	395	356	326	321	374	354	248	301	274	236	286	269
2	258	297	374	384	356	334	297	305	248	268	274	278
3	358	367	314	325	387	350	247	295	287	263	248	268
4	368	347	315	348	367	349	274	235	308	247	278	268
5	374	310	359	362	348	351	263	310	258	267	265	273
						348						271
No. of capillary	**K₁₁**	**K₁₂**	**K₁₃**	**K₁₄**	**K₁₅**	t_{tw}^{o}**2**	**K₁₆**	**K₁₇**	**K₁₈**	**K₁₉**	**K₂₀**	t_{tw}^{o}**3**
Cycle No.												
1	236	247	256	284	296	264	289	301	305	274	268	287
2	239	254	261	247	254	251	258	234	314	287	277	274
3	301	278	241	251	236	261	265	287	307	254	295	282
4	270	265	248	264	275	264	274	269	268	283	298	278
5	263	258	247	302	279	270	281	269	275	297	306	286
						262						281

Table 8. *The reaction of white mice to extract sample No. 1*

Extraction day	White mouse mass, g	Exposure time, hour	Reaction
1	19	2	Negative
2	20	2	Negative
3	20	2	Negative
4	19	2	Negative
5	19	2	Negative
6	21	2	Negative
7	20	2	Negative
8	19	2	Negative
9	20	2	Negative
10	20	2	Negative

Table 9. *The reaction of white mice to extract sample No. 2*

Extraction day	White mouse mass, g	Exposure time, hour	Reaction
1	20	2	Negative
2	20	2	Negative
3	22	2	Negative
4	19	2	Negative
5	21	2	Negative
6	21	2	Negative
7	20	2	Negative
8	19	2	Negative
9	19	2	Negative
10	20	2	Negative

Table 10. *The reaction of white mice to extract sample No. 3*

Extraction day	White mouse mass, g	Exposure time, hour	Reaction
1	22	2	Negative
2	22	2	Negative
3	20	2	Negative
4	18	2	Negative
5	19	2	Negative
6	19	2	Negative
7	20	2	Negative
8	20	2	Negative
9	21	2	Negative
10	22	2	Negative

Table 11. *The distribution of patients by disease*

Diseases	Quantity	Specific gravity, %
Posthemorrhagic anemia	5	20
Anemia in GIT chronic diseases	16	64
Anemia in chronic kidney diseases	4	16

Table 12. *The distribution of patients by age*

Age	Quantity	%
15-25 years	7	22
26-45 years	18	58
46-70 years	5	20

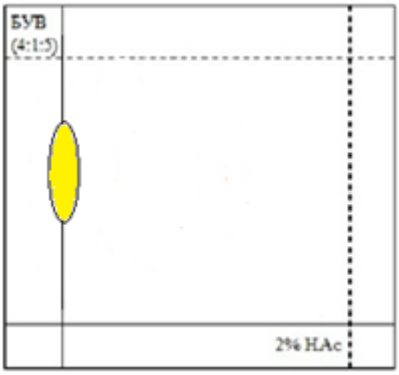

Figure 1. *Aglycon Chromatogram*

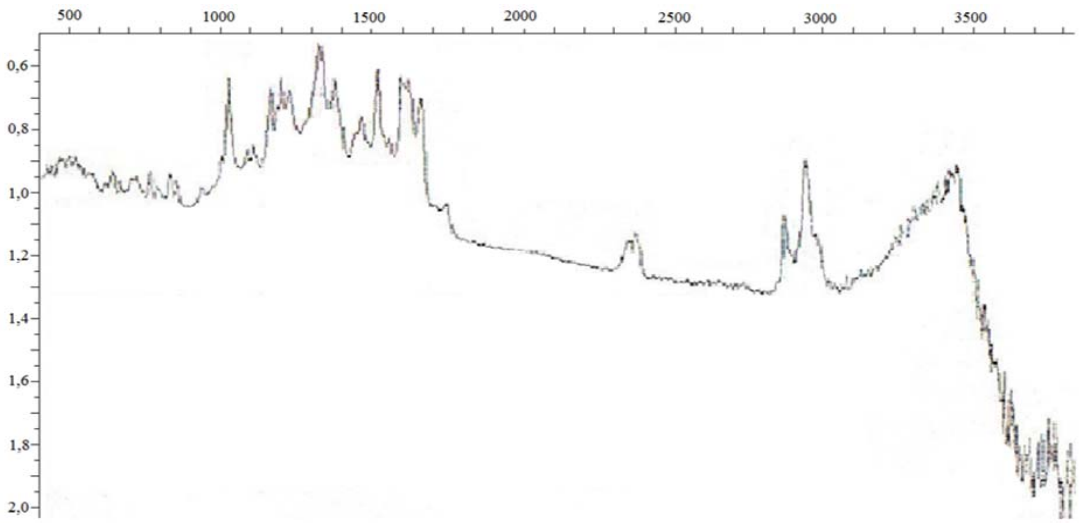

Figure 2. *IR spectrum of myricetin fractions from the Linosyris villosa plant*

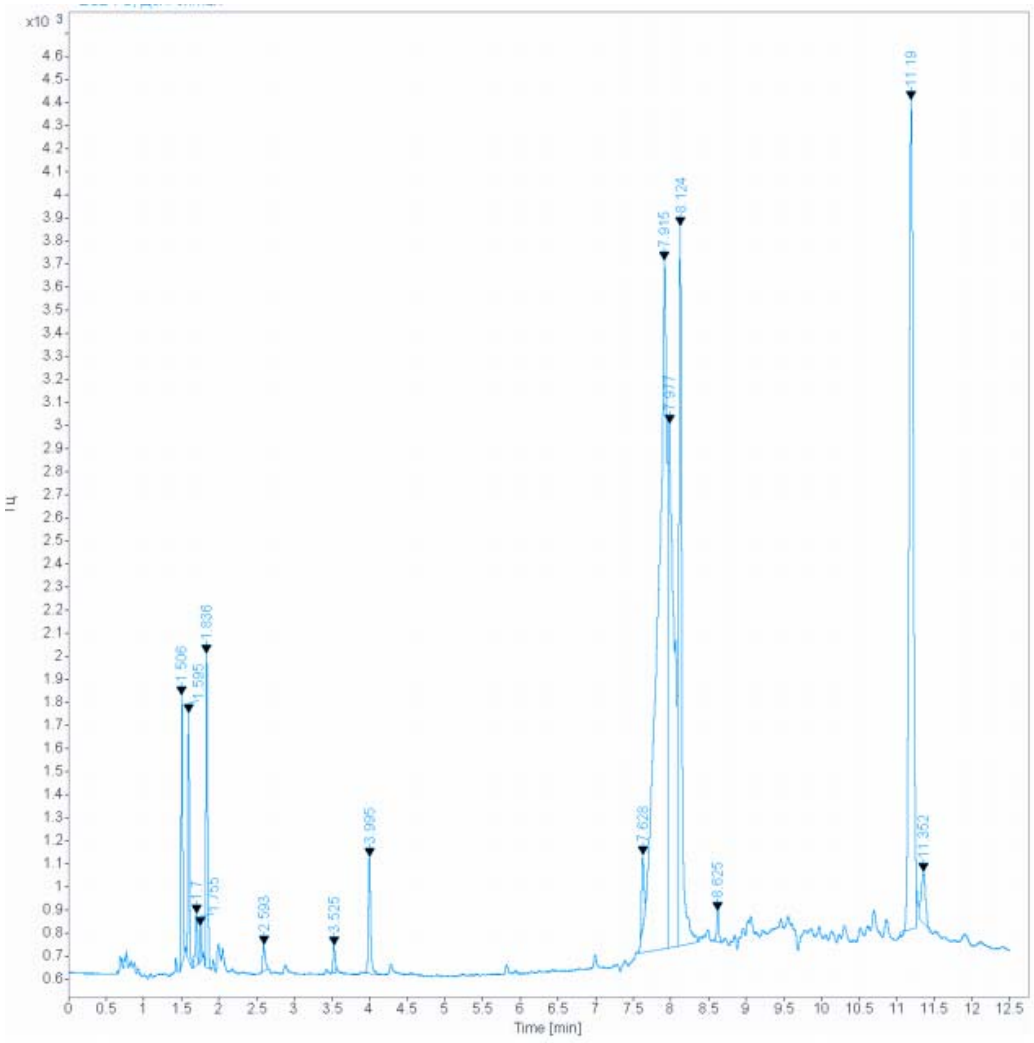

Figure 3. *The chromatographic spectrum of the studied extract (Sample_3)*