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EXTRACTION OF QUERCETIN FROM *LINOSYRIS VILLOSA*

Abstract. The chemical composition of the plant Linosyris villosa is being investigated for the first time. The following ingredients were extracted from this plant: aglikon quercetin of phlavonoid nature. Different types of chromatography have been applied (column adsorbing, preparational); there was extracted an individual form of quercetin. The physical-chemical methodology analysis was used to prove its structure.

Keywords: quercetin, aglikon, plant Linosyris villosa, chromatography, column, preparational, biological activity, antioxidental, immune-modeling, antibiotic.

Introduction. Flavonoids take active part in the cellular circuit processes of a plant. They function as growth regulators, as well as plant development and reproduction regulators. Scientists are interested in flavonoids due to the wide spectrum of their biological activity. A lot of phytopreparations contain flavonoids. While being the safest medicines, these preparations are paid close attention to. Plants contain varied amounts of flavonoids: from 0.5% to 20% (e.g. blossoms of *Styphnolobiumjaponicum*) in average. Flavonoids contained in plants can be of two types: aglycones or glycosides. The *Embryophyta*, such as the family *Compositae* (*Asteraceae*), *Polygonaceae* and legumes, are especially rich in flavonoids.

Flavonoid preparations can potentially help to prolong human life, as some of them have anti-sclerotic and anti-oxidant activities, which slow down aging processes. In a human body, flavonoids influence both enzymatic systems and immune processes, causing various effects. A lot of scientists assert that the wide spectrum of biological activity of flavonoids is predetermined by their anti-oxidant activity. Flavonoids, such as quercetin, myricetin and kaempferoland rutin, can not only bind, but also restore or oxidize ions of metals with *variable* valencies. By this way they are able to stimulate or inhibit free radical processes occurring in a human body [1].

The interconnection between the structure and anti-oxidant activity has been studied for most of the flavonoids produced by plants. It has been found out that flavonoids, as well as polyphenols, can serve as a ‘trap’ for free radicals and inhibit peroxidation of lipids. The most active flavonoids are quercetin, myricetin and morin which inhibit lipid oxidation from 78 to 83%. Being antioxidants the flavonoids play an important part in protecting the structure and functions of the liver in cases of pathologies, quicken the regeneration and restore the functional activity of hepatocytes, especially in cases of complex therapy of acute and chronic hepatitis and liver cirrhosis.

In the modern medicinal practice, flavonoid preparations are widely used in various forms: pills, ointments, tinctures, extracts, powders, dragees and capsules. Much attention is paid to the anti-inflammatory activity of flavonoids which is probably connected to their antiulcerant, wound healing, antipyretic and astringent activities.

The antimicrobial properties of flavonoids are also remarkable. One has detected the negative influence of quercetin and myricetin upon gram-positive bacteria, as well as the influence of flavones and chalconoidsupon staphylococcus. Gallocatechin, epigallocatechin and the oxidized sum of catechinshave antimicrobial properties active for staphylococci and streptococci. Isoflavones, isoflavonoids and flavonolsare chemically and biochemically remarkable. All of them have cholesteric, diuretic and antihyperglycemic activity in various degrees. The large therapeutic potential of flavonoids allows to regard them as sources of general treatment activity.

Flavonoids have a valuable property of quick evacuation and the absence of cumulation. Flavonoid preparations are essential not only for treating illnesses, but also for the prevention of vascular problems. Flavonoid preparation treatment has proved effective in cases of cavitary diseasein the post-operation period, glaucoma and hyperthyroidism [2].

The anti-oxidant activity of flavonoids has been studiedrecently. In this regard, the problem of creating medicines with anti-oxidant properties has become topical. Such preparations can be used for the prevention and cure of illnesses which are accompanied by the intensification of free radical reactions. Bioflavonoids are ranked first among exogenous natural antioxidants. The most pharmacologically remarkable are preparations with the high proportion of aglycones and glycosides, such as quercetin, kaempferol, apigenin, luteolin, isorhamnetin and methoxylated 6-oxyflavonols [3].

Such pharmaceutical dosage forms containing quercetin as *Corvitin* and *Lipoflavon* are rather effective in various pathological conditions. The therapeutical effect of using soluble and injection forms become apparent significantly quicker. Due to the wide spectrum of the physiological activity of quercetin, the creation of such forms are of special importance.

Ukranian scientists have created a new pharmaceuticaldosage form – *Corvitin* – which is water-soluble powderfor preparing injections on the basis of the synthetic modulator of solubility (polyvinylpyrrolidone). *Lipoflavon*has been created with the help of phosphatidylcholineliposomes. The use of phosphatidylcholineliposomes as carriers allows to inject the preparation into blood. It is possible due to the penetration through the phospholipidbilayer of membranes and the quick transportation of the preparation to the target cells.

As a result of the preclinical trial, it has been found out that *Corvitin*has low toxicity indicators. It does not possess any allergic influence. It has been detected that *Corvitin*has a significant anti-oxidant effect, as well as an inhibiting activity for membrane enzymes, especially lipoxygenases, activating and preserving the

nitrogen oxide level in the affected tissues and blood, protecting membrane-bound enzymes which regulate ion (calcium) homeostasis in the cells.

The wide spectrum of the pharmacological characteristics of *Corvitin*has been a major cause for its further testing. Especially significant activities of this preparation are anti-oxidant, anti-inflammatory and membrane-stabilizing actions which are predetermined by the inhibiting influence upon the essential enzyme systems of a cell.

Tests have shown that *Lipoflavon*in the 0.1 mg/kg dosage with the parenteral route of administrationhas a positive effect on the nerve fiber regeneration. Both the number of nerve fibers and the regeneration rates are increased. The therapeutic effect of *Lipoflavon*is predetermined by the liposome form, which is a product of nanotechnology having high affinity rate to the cell membranes, and quercetin, which is an antioxidant protecting nerve cells from the oxidation stress, activating endogenous anti-oxidant protection systems, reducing the inflammation and increasing the axon growth. It contributes to the regeneration processes of the nerve fibers and can prevent degradation processes at the late stages of restoration.The liposome form of quercetin accelerates the emergence and myelination of nerve fibers. Even within a short period of usage (10 days) *Lipoflavon*has an apparent neuroprotective effect [4].

Quercetin is one of the most well-known and profoundly studied flavonols. It is wide-spread in the plant kingdom. The term ‘quercetin’ stems from the Latin word ‘quercus’, which means ‘oak’. Both oak bark and oak timber contain this substance. The biggest amount of quercetin is in tea (up to 2500 mg/kg of dry leaves). Quercetin belongs to the Vitamin P group. It is found in onions, apples, blueberries, black and green tea, red wine, leafy green vegetables and legumes. For industrial (pharmaceutical) needs, it is extracted by hydrolysis of rutin which is produced from *Styphnolobium japonicum* or buckwheat. Organic fruits and vegetables contain much more quercetin. Thus, tomatoes which have been grown in the natural environment, for instance, contain 79% more of this useful flavonoid than hothouse tomatoes.

Quercetin can be taken as a food additive. However, it is badly digested as a separate substance – most of it is metabolized into inactive phenolic acids or just gets removed from the human body. Nutrients are always better assimilated from whole foods rather than from their separate fragments.This phenomenon can be explained by the fact than any substance naturally exists in a combination with a variety of other synergizing nutrients which increase their assimilation and utilization rates.

By now, there has been extensive research of quercetin preparations for the possibility of their use in order to prevent and cure various illnesses. The summary of the sources available shows a wide spectrum of biochemical and pharmacological properties of quercetin [1, 2].

It is believed that quercetin possesses a wide spectrum of biological activity. It can positively influence metabolism, thus, preventing obesity. It can also

demonstrate an anti-inflammatory effect and prevent atherosclerosis. It is also capable of blocking tumor cell proliferation, decreasing the risk factor expression of cardiovascular diseases. It is regarded as an agent which can inhibit the progress of atherosclerosis-related processes.

Having the ability of inhibiting the activity of 5-lipoxygenase, quercetin demonstrates anti-inflammatory properties and synergism with nonsteroidalanti-inflammatory preparations. The ability to block cell division makes quercetin useful both as an anti-inflammatory preparation and as a cancer prevention means [3].

Quercetin neutralizes aggressive oxygen-containing and nitroxyl radicals, breaks the reaction chains of free radicals. Hence, it can stop pathologic processes in cells. Oxidation products of free radicals are the main agents of the oxidation stress in the cell. They cause various illnesses. Thus, the prevention of free radicals' formation or their neutralization is essential for the successful treatment of illnesses.

The combination of anti-oxidation and membrane-stabilizing properties of quercetin contributes to the decrease in capillary permeability and stabilization. As a result, the increase in energy supply of cardiac hystiocytes due to the anti-oxidation activity and enhanced blood flow predetermines the cardioprotective effect of quercetin.

The selective inhibiting influence upon cell enzymes also lies in the basis of pharmacological and biochemical effects of quercetin. It allows to position quercetin as a specific bioregulator of enzymatic processes occurring in the human body.

Numerous recent researches has proved that quercetin has neuroprotective, anti-oxidation, immunomodulatory, membrane-stabilizing, cardioprotective, antihypoxicand anti-inflammatoryactions, it reinforces reparative processes in the organism. Experimenting on volunteers has shown that quercetin is able to produce a positive effect upon patients suffering from inflammatory or oxidation stress, but it does not show any considerable effect when it is used by healthy people [4].

EXPERIMENT

The plant *Linosyrisvillosa* has been used in the given research. Samples were gathered in its blooming phase in the North-Kazakhstan Oblast.The raw material was dried and crushed till the air-dry state. Then pharmacopoeia indicators were tested. The moisture content in the plant was equal of 8.1% [5].

The raw material was extracted by hexane in the Soxhlet extractor, in the water bath, with the hexane boiling temperature for 10 hours. The ethanolic extract was boiled dry. The dark brown sediment was dissolved in ethanol and then it was applied to the column with aluminium oxide.

Two zones were observed on the column: the upper zone was black, and the lower one was yellow.The elution of the yellow zone was conducted by

96%-ethanol. Three fractions (5 ml of each) were gathered. All fractions were studied with the use of the two-dimensional paper chromatography method in the dissolvent system of butyl alcohol – acetic acid – water with the 4:1:5 ratio (I) and 2%-acetic acid (II).On the chromatogram of the first fraction, there was an oblong spot which had the values Rf (I) = 0.80 and Rf (II) = 0.00. In the ultraviolet (UV) light, the spot fluoresced showing the yellow colour. In the ammoniavapour, it acquired the bright yellowcolour. This fact shows that it is an aglycone of the flavonoid nature. Because the spot was oblong, it was assumed that the chromatogram contained several flavonoid aglycones [6].

The chromatogram of the second fraction also contained an oblong spot. In the UV light, the spot fluoresced showing the yellow colour. In the ammoniavapour, it acquired the bright yellow colour. The position of the spots on the chromatograms of the first and second fractions indicates that the spots are flavonoid aglycones with the values Rf (I) = 0.80 and Rf (II) = 0.00. The chromatogram of the third fraction contained a spot with Rf (I) = 0.78 and Rf (II) = 0.00, which also corresponds to a flavonoid aglycone. In the UV light, the spot fluoresced showing the yellow colour. In the ammoniavapour, it acquired the bright yellow colour. The substance on the third chromatogram was identical to quercetin [7, 8].

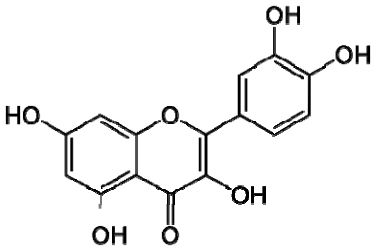
The eluates of Fractions 1 and 2 with Al_2O_3 were combined and boiled. An oily mass was produced which was further processed with distilled water. Then the sediment was dissolved in 96%-ethanol and further chromatographed on the column with aluminium oxide. On the column, there was one yellow zone which was eluated by the mixture of ethanol and chloroform with the ratio 9:1. The produced eluate was yellow. It was boiled dry and crystalized from 80%-ethanol solution [9].

As a result, an amphoteric substance of yellowish brown colour with the fusion temperature of 288-290 ºC was produced.

The method of preparation chromatography was used for the further purification of the substance. The dissolvent system of butyl alcohol – acetic acid – water with the 4:1:5 ratios was used and 86 mg of the substance was produced. Aglycone was crystalized from ethanol. A substance of yellowish green colour with the fusion temperature of 304-308 ºC was produced. Aglycone was studied with the help of the two-dimensional paper chromatography method in the dissolvent system of butyl alcohol – acetic acid – water with the 4:1:5 ratio (I) and 2%-acetic acid (II). On the chromatogram, there was a spot which had the values Rf (I) = 0.80 and Rf (II) = 0.00. In the UV light, the spot fluoresced showing the yellowish colour. In the ammoniavapour, it acquired the bright yellow colour. According to the position of the spot on the chromatogram and its colour, the substance was identified as an aglycone of the flavonoid nature.

The infrared (IR) spectrum has been identified for the aglycone using KBr. There are absorption bands in the IR spectrum, in the area of 1,660 sm^{-1}, which correspond to the vibrations of the carbonyl group (C=O); in the area of 3,450 sm^{-1}, which correspond to the vibrations of the hydroxyl groups(-OH); in the area of 2,850, 2,940 sm^{-1}, which correspond to the vibrations of the (C=C) aromatic ring.

Conclusion. Hence, according to the fusion temperature, qualitative reactions, data sources and IR spectroscopy, the produced aglycone has been identified as quercetin with the following structural formula (figure) [10].



Stuctural formula of Quercetin

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Резюме

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LINOSYRIS VILLOSA ӨСІМДІГІНЕН КВЕРЦЕТИНДІ БӨЛІП АЛУ

«Lynosyris villosa» өсімдігінің химиялық құрамы алғаш зерттелуде. Өсімдіктен флавоноидты табиғатты кверцетин агликонын бөліп алдық. Хроматографияның әр түрлерін: бағаналық, адсорбциялық, препаративті қолдана отыра, кверцетинді жеке түрде бөліп алып, оның құрылысын талдаудың физика-химиялық әдістерімен дәлелдедік.

Түйін сөздер: кверцетин, Lynosyris villosa өсімдігі, хроматография, бағаналық, препаративті, биологиялық белсенділік, антиоксидантты, иммуномоделирлеуші, қабынуға қарсы.

Резюме

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ВЫДЕЛЕНИЕ КВЕРЦЕТИНА
ИЗ РАСТЕНИЯ *LYNOSYRIS VILLOSA*

Химический состав растения «Lynosyris villosa» изучается впервые. Из растения выделили агликон-кверцетин флавоноидной природы. Применяя разные виды хроматографии, колоночную, адсорбционную, препаративную, выделили кверцетин в чистом виде и доказали его строение физико-химическими методами анализа.

Ключевые слова: кверцетин, растение Lynosyris villosa, хроматография, колоночная препаративная, биологическая активность, антиоксидантная, иммуномоделирующая, противовоспалительная.