STUDY OF FLAVONOIDS ON THE SURFACE OF SCUTELLARIA LEPTOSIPHON PLANT

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Article History: **Received:** 17.02.2023 **Revised:** 07.03.2023 **Accepted:** 20.03.2023

Abstract

Aim: Scutellaria leptosiphon Juz. It consists in studying the flavonoids of the plant and determining their chemical structure.

Methods: to extract flavonoids from the plant, we extracted the plant in alcohol and butanol and divided the obtained extractions into fractions. We diluted the alcohol obtained as a result of extraction with distilled water and extracted it with ethyl acetate in a separating funnel. As a result, we separated the fraction with ethyl acetate. In the approximate determination (identification) of the number of flavonoid compounds in the extracts prepared from plants and their authenticity, the distribution (separation) chromatographic method (on paper - PC and a thin layer - TLC) was widely used. Spectrophotometric methods are used to determine the number of flavonoids in the product. We used the photoelectric colourimetric method in the laboratory.

Results: S.leptosiphon plant was first collected from the Urgut mountains and its above-ground part was studied it was found that it contains Norvogonine flavone and its glycoside.

Conclusions: Scutellaria leptosiphon Juz. practical works such as extraction, separation into fractions in solvents, paper chromatography, thin layer chromatography, and qualitative reactions were performed to extract flavonoids from the surface of the plant. The composition of the sum separated with butanol Norvogonin 7-O-β-D-glucopyranoside was found. The substances obtained as a result of the experiments gave the results of the qualitative chromatographic analysis by the test substances, and it was the basis for our conclusion that their structure is the same as these substances. We believe that the number of substances contained in the studied plant extract, the adequacy of the raw material base, and the high pharmacological activity of this plant in the future, can be used in the field of pharmaceuticals.

Keywords: Scutellaria, Scutellaria leptosiphon, flavonoids, norvogonin, paper, and thin layer chromatography.

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DOI: 10.31838/ecb/2023.12.1.041

I. Introduction

1.1. Relevance of the topic

Humanity, in its daily life, uses six thousand of the five hundred thousand plant species in the world. 1500 of them are among medicinal plants. Today, a lot of research is being carried out on extracting medicinal substances from plants, determining their chemical structure and biological activity, and applying them to practice. In scientific medicine, 45% of medicinal preparations are extracted from plants. In modern medicine, the demand for natural medicinal products obtained from plants is increasing, because no matter how fast and effective the drugs obtained by synthesis are, their long-term continuous consumption can cause various unpleasant effects in the living organism causing changes to occur [1,2,3]. Therefore, extracting physiologically active substances from plants, synthesizing new derivatives, and creating effective new drugs for medicine are important tasks in this field.

Today, worldwide attention is being paid to the study of the chemical composition of plant species rich in biologically active substances. These include plants belonging to the Scutellaria L. family, the chemical composition of more than 65 species of them was studied, and phenol carboxylic acids, phenylpropanoids, iridoid glycosides, diterpenes, flavonoids, lignans, other natural compounds were isolated from them. Several substances such as lacrizid, lespeflan, flacarbin, flakumin, rutin, liquiriton, datiscan, flamin, and silibor are widely used in medicine as effective medicinal agents.

Extensive measures are being taken in our country to create import-substituting natural medicines based on local medicinal plants and to provide the population with quality medicines. In particular, scientific research is being carried out to analyze the chemical composition of Scutellaria L. species growing in the territories of the Republic of Uzbekistan, to extract flavonoids from these plants, and to study their chemical structure and pharmacological activity. In the 4th direction of the Strategy of Actions for the further development of the Republic of Uzbekistan, important tasks are defined in the context of "Further development of the pharmaceutical industry, improvement of the provision of affordable, high-quality medicines and medical supplies to medical institutions and the population." Based on these tasks, it is important to isolate biologically active compounds from plants, to determine their chemical structure, and at the same time to accelerate the work in the direction of creating medicines based on them, to create new effective medicines from local raw materials.

Resolution of the President of the Republic of Uzbekistan No. PF-4947 of February 7, 2017 "On the Strategy of Actions for Further Development of the Republic of Uzbekistan", No. PF-5229 of November 7, 2017 "Management of the Pharmaceutical Network These results are of practical importance in the implementation of the tasks defined in the Decree on measures to radically improve the system and other regulatory legal documents related to this direction [4].

1.2. Level of study of the problem

The chemical composition of more than 65 species of Scutellaria plants was studied, and more than 330 phenolic substances were isolated from them. Foreign scientists Y.Imoto, H.Kizu, T.Namba, N.Joshee, Y.Y. Zhang, C.R. Yang, Z.H. Zhoi, J. Miao, T. Tomimori, S. Shibata, Y. Kikuchi, Y. Miaichi, I.I. Chemesova, D.I. N.K.Chirikova, V.I.Litvinenko, T.P.Popova, M.Iinuma, A.L.Budansev, and others conducted scientific research. Baicalin, baicalein wogonin, and other substances extracted from Scutellaria plants have antiviral and antibacterial properties and are effective drugs against inflammation, AIDS, cancer, and seizures.

V.M. Malikov, E.Kh.Botirov, SH.V.Abdullaev, M.P.Yuldashev, R.Muradov, F.D.Nasrullaev, A.M.Karimov, K.A. Eshbakova, and others conducted research in this direction in Uzbekistan. Many new and known flavonoids were isolated by these scientists from plants belonging to the Scutellaria L. family, the structure of the obtained substances was scientifically proven and their pharmacological activity was determined. Based on these, continuing research on new species of Scutellaria L. is an urgent, scientific-practical topic [5-26].

1.3. The goals and objectives of the research

For the first time, flavonoids of the S. Leptosimum plant belonging to the Scutellaria family growing in the Samarkand region were studied.

The above-ground part of the S. leptosiphon plant belonging to the family Scutellaria was collected and dried in the shade, then crushed. Methods such as extraction of plant raw materials using solvents such as ethyl acetate, butanol, and separation into fractions close to each other in terms of polarity were used to separate substances from complex mixtures. Scientific research is being carried out at plant science centres and botanical institutes to examine the plant belonging to the Scutellaria family.

Among them, chemical investigations are being conducted at Namangan State University, Institute of Chemistry of Plant Substances, Bioorganic Chemistry under the leadership of Sh.V.Abdullaev. It was identified by Japanese scientists V. I. Litvinenko, Y. Kikuchi, T. Tomimori. We can cite the following as evidence for our opinion. Baicalein, scutellarin, norvogonin, metaholin, wogonoside, chrysin 7-glucuronide arabinoside, and similar flavonoid substances were isolated from the above representatives of broccoli plants as a result of chemical analysis. Based on these results, flavonoids can be used as effective drugs that dissolve well in water and as dyes.

1.4. The scientific novelty of the research. For the first time, the flavonoids contained in the above-ground body parts of S. Leptosiphon, a member of the Scutellaria plant family, were studied. To study its flavonoids, it was extracted in solvents such as butanol and ethyl acetate. Sums were extracted from the extracts and analysed using paper chromatography (PC) and thin-layer chromatography (TLC).

1.5. The scientific and practical significance of the research

With the creation of synthetic and chemical methods of obtaining medicine, various new compounds that do not occur in nature, including medicinal substances, began to be synthesized. In general, there have been many cases of negative effects on the human immune system of synthetically created medicinal preparations that are not fully compatible with the human body. Proper use of medicinal plants in the territory of our republic, identification, isolation, analysis of the physiologically active substances contained in them, and the study of their pharmacological effects are important.

1.6. Chromatographic analysis of flavonoids

In the approximate determination (identification) of the number of flavonoid compounds in the extracts prepared from plants and their authenticity, the distribution (separation) chromatographic method (on paper - PC and a thin layer - TLC) was widely used. An alcoholic extract is prepared from the plant for chromatographic analysis. To do this, put 1 g of the above-ground part of the plant in a 25 ml flask and pour 10 ml of alcohol over it. Place a cooler in the flask in an upright position and boil it in a water bath for 10 minutes.

After cooling, the solution is filtered through a paper filter. 0.1 ml of the filtrate and alcohol solution of "witness" flavonoids are dripped onto the starting line of the "Silufol" plate using a capillary tube at a distance of 2 cm from each other and dried in the air. Then the plate was placed in a chromatographic column filled with n-butanol - acetic acid - water (4:1:5 ratio) or a 15% solution of acetic acid and chromatographed for 30-40 minutes.

Then the plate was taken out and dried in the air, and the spots were determined by looking at the UV light (flavonoids are dissolved in brown, yellow, and golden colour). Then, an alcohol solution of aluminium chloride (or zirconium chloride oxide, iron (III)-chloride solutions) is sprayed on the plate, dried, and seen again under UF light. These PCs were compared with the "witness" flavonoid PCs, and it was thought about what flavonoids are present in the plant extract.

Chromatographic analysis can be performed on paper using the same method.

In addition to determining whether flavonoids are present in separations or chromatograms using the above and other qualitative reactions, it is possible to determine which carbon atom the hydroxyl groups are located in the flavonoid molecule and whether these groups are pure or combined with a sugar molecule. For this, according to the scheme recommended by Prof. V.A. Bandyukova (Pyatigorsk Pharmaceutical Institute), paper chromatograms were subjected to qualitative reactions using Wilson and Martini-Bettolo reagents, zirconium chloride oxide, diazo reagent, and other reagents.

1.7. Determining the number of flavonoids in plants

There are many different ways to determine the number of flavonoids in plants. Determination of the number of flavonoids in the product is mainly by spectrophotometric methods. But taking into account that the spectrophotometer is still not enough in all laboratories and departments, it is necessary to describe here the photoelectric colourimetric method, which is quite simple to perform at the moment.

After combining 1 g of dried and crushed product with 100 ml volume and a vertical cooler, add 30 ml of chloroform to it and heat it in a water bath for 5 minutes. Then the chloroform extract is filtered. After adding 30 ml of chloroform to the product, it is extracted 2 times in the same way as before. Since tar, chlorophyll, and similar unnecessary ballast substances are released in the chloroform extract, this extract is discarded.

The product in the flask is heated and dried at 50-60 ℃ until it is cleared of chloroform. Then, to extract flavonoids from the product, 30 ml of methyl alcohol (methanol) is added to the flask, the flask is connected to a vertical cooler, and the mixture is boiled in a water bath for 30 minutes. After the specified time, the flask is cooled, and the flavonoid extract (extract) is placed in a 50 ml measuring flask. The product in the flask is rinsed with methanol, placed in a volumetric flask containing the extract, and filled with methanol until the volume of the liquid reaches the mark of the volumetric flask.

The liquid in the measuring flask is mixed and filtered to obtain the extract (extract A) needed to determine the number of flavonoids. The amount of flavonoids in the extract is determined by the photo-colourimetric method. This method is based on the colour reaction of flavonoids with the novocaine (or sulfonyl acid) diazo compound. For this, mix 1 ml of 0.5% solution of novocaine dissolved in 10% sulfuric acid and 1.5 ml of 0.2% sodium nitrite solution in a 10 ml measuring flask. 2 ml of extract A and 1 ml of 10% solution of sodium hydroxide are added to the mixture, and the liquid volume is filled with methanol up to the mark of the measuring flask. Then the liquid in the

flask is mixed and the colour intensity is measured using a photoelectric colourimeter in a 1 cm thick cuvette with a blue light filter.

The concentration of flavonoids in extract A was found using a graph based on a standard solution (rutin, quercetin, or other pure flavonoid solution).

The percentage of flavonoids in the product (X) is calculated using the following formula (1):

$$x = \frac{\alpha * 10 * 50 * 100 * 100}{2 * c(100 - v)} \quad (1)$$

where α is the concentration of flavonoids in 1 ml of extract A;

v - product moisture (in %);

c - the amount of the analyzed product in grams.

To extract flavonoids from the plant, we extracted the plant in alcohol and butanol and divided the obtained extracts into fractions.

We diluted the alcohol obtained as a result of extraction with distilled water and extracted it with ethyl acetate in a separating funnel. As a result, we separated the fraction with ethyl acetate.

II. Practical Part

2.1. Plant selection and preparation

Most plants contain biologically active substances and have healing properties that are used for the treatment of various diseases. Our region is rich in such plants.

It is known that the blueberry plant is of special importance due to its richness in medicinal substances. In Uzbekistan, 32 species of the blueberry plant have been identified, of which the composition of about 60 species around the world has been studied, and more than 200 medicinal and physiologically active substances, flavonoids and their glycosides, have been isolated from them.

It has been determined based on experiments that the chemical composition of the plants of the genus Cucamaron contains a large number of types and amounts of flavonoids and their pharmacological effects.

The obtained results are consistent with the results of the paper and thin layer chromatography, and qualitative and quantitative analysis, and our opinion was reflected in the literature. We have achieved good results by using a non-traditional solvent sequence in the extraction of flavone glycosides from the above-ground part of the blueberry plant.

The above-ground part of the plant was harvested in the autumn months after it stopped growing. The above-ground part of the harvested plant was cleaned from various mixed plants and soil, washed in cold water, and then we focused on drying the plant, which is the main part of our work.

The surface of the plant, dried at 50-60 ℃, was ground into powder by mechanical grinding in a mortar.

2.2. Plant extraction

200 g of crushed roots of the dried Scutullaria leposiphon plant were placed in a 1-litre extraction flask and 0.6 litres of gasoline was poured over it. After leaving for a day at room temperature, the first fraction was isolated on a paper filter. Then we poured another 0.4 litres of extraction gasoline on the plant and left it for a day. Then we filtered the second fraction in the same order as the first fraction. This work was carried out five times from 0.4 litres each time until the colour intensity practically disappeared, and 2 litres of extraction gasoline fraction was separated. The remaining plant was dried and put back into the extraction flask.

The surface part of the plant cleaned with extraction gasoline was continued to be extracted with 80% ethanol, and 0.4 litres were extracted 6 times to obtain 2.4 litres

of ethanol extract. The obtained extract was evaporated using a rotary evaporator and we put it in a porcelain container and evaporated it. Because ethanol does not interfere with working with other solvents, it was completely evaporated, and 20 grams of the thick sum was separated.

2.3. The division into factions

The obtained 20 g of alcohol was diluted with distilled water in a ratio of 1:1, and the fraction with ethyl acetate was extracted 6 times from 50 ml with ethyl acetate in a separatory funnel, and when this fraction was evaporated, 6 g of ethyl acetate were obtained.

When we extracted the aqueous alcohol mixture in the remaining separation funnel with H-butanol in the above procedure, 500 ml of butanol extract was obtained. The obtained extract was evaporated in a water bath in a rotor evaporator, and 5 g of butanol was isolated. The obtained amounts were further analyzed using thin-layer chromatography (TLC) and paper chromatography (PC).

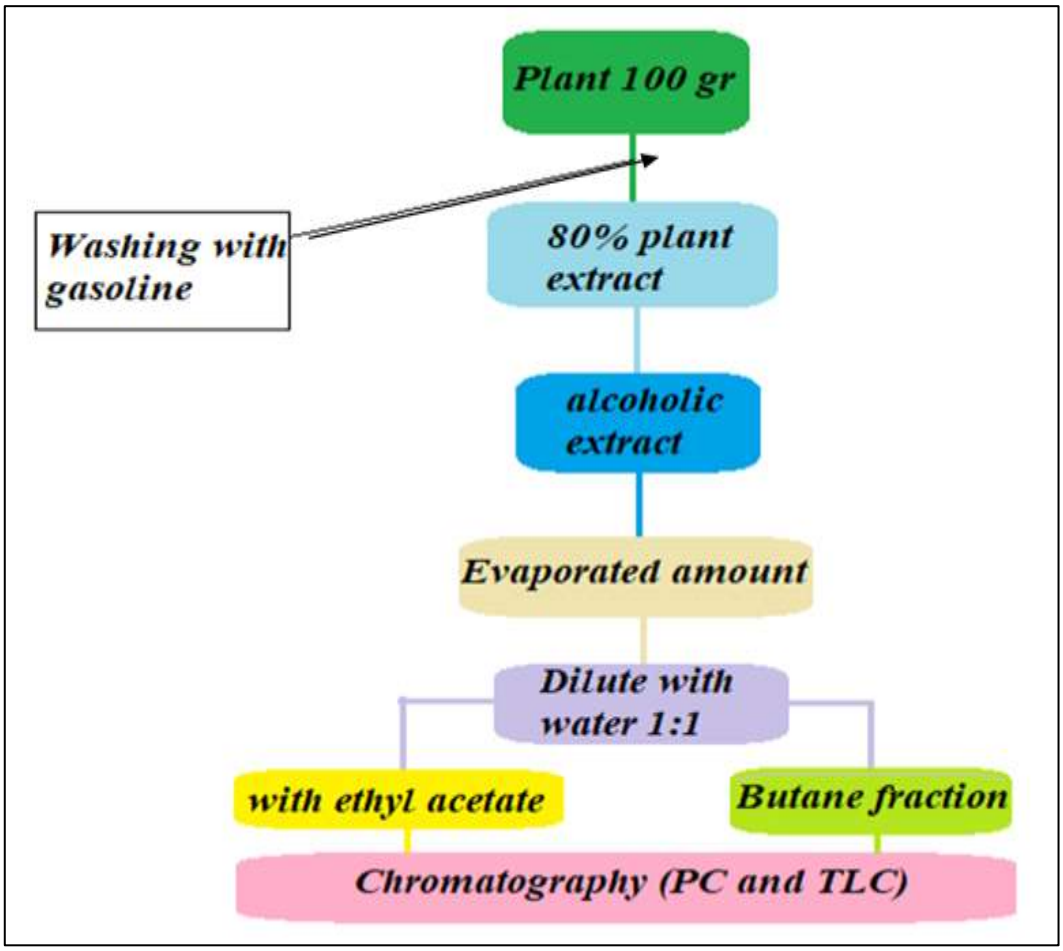

Fig. 1. Double chromatography

2.4. Examination of aggregates using chromatographic analysis

Table 1. Ethyl acetate sum chromatography results

Solvent chloroform-methanol (4:1) TLC and PC

Substance №	With normal light	NH_3 when the solution	Ultraviolet rays	$AlCl_3$ when the solution	Ultraviolet rays	alcoholic solution of NaOH)	Ultraviolet rays	Rf

		is sprayed		is sprayed		when sprinkled		
1	-	-	Pale yellow	-	Pale yellow	-	Yellow	0.80
2	-	-	Yellow	-	Yellow	-	Yellow	0.65
3	Brown	Dark brown	Blackish	Dark brown	Blackish	Dark brown	Blackish	0.52
4	-	-	Orange	Open Yellow	Brown	-	Yellow	0.40
5	-	-	Bright pale yellow	-	Purple colour	-	Bright pale yellow	0.20

Table 2. Ethyl acetate sum chromatography results
Solvent chloroform-methanol (9:1)

Substance №	Normal light with	NH_3 when the solution is sprayed	Ultraviolet rays	When $AlCl_3$ is sprinkled	Ultraviolet rays	NaOH When sprinkled with the alcohol solution	Ultraviolet rays	Rf
1	Brown	Brown	Dark brown	Dark brown	Blackish	Brown	Dark brown	0.67

Table 3. Butanol sum chromatography results
Solvent chloroform-methanol (4:1)

Substance №	With normal light	NH_3 When sprinkled	UB	$AICI_3$ When sprayed	UB	NaOH When sprinkled	UB	Rf
1	-	-	Bright pale yellow	-	Pale yellow	-	Pale yellow	0.58
2	-	-	Pale yellow	-	Purple colour	-	Yellow	0.25
3	Pale yellow	Yellow	Orange	Yellow	Yellow	Yellow	Orange	0.08

Table 4. Butanol sum chromatography results
Solvent chloroform-methanol (9:1)

Substance№	With normal light	NH_3 when the solution is sprayed	Ultraviolet rays	When the $AlCl_3$ solution is sprayed	Ultraviolet rays	NaOH when sprinkled with the alcohol solution	Ultraviolet rays	Rf
1	Yellow	Yellow	Yellow	Yellow	Orange	Yellow	Yellow	0.67

To identify the tested components in the chromatogram, the distribution coefficient Rf of the substances in the used solvent system is used. Rf is calculated as follows: for this, the distance from the drop point (start) to the centre of the spot (x) is divided by the distance from the start line to the solvent front line (y):

$$R_f = \frac{x}{y} \quad (2)$$

The determined value of Rf is compared to the table for pure substances to which substance it corresponds. But since the value of Rf depends on the used system, temperature, type of paper and other factors, the chromatographic analysis was carried out with the participation of certain substances - "witnesses".

When examining the mixtures of some substances, it is not possible to obtain an appropriate result by performing chromatography in one direction. In such cases, chromatography is performed once, the obtained chromatography is rotated by 90°, and chromatography is performed a second time (Fig. 1). This method is called double chromatography. Recently, thin-layer chromatography, especially gas-liquid chromatography, is widely used among the more convenient and fast types of chromatography.

2.5. Checking the extract using qualitative reactions

2.5.1. Reaction with ammonia.

When we added ammonia solution to the alcohol solution of flavonoids obtained in a porcelain container and heated it a little in a water bath, as a result of the reaction, the solution of flavones, flavonols, and flavanones produced a yellow colour that turns golden or red. The addition of ammonia solution to the solution of chalcones and aurones produced a red or dark red colour without heating. Anthocyanins were coloured purple under the influence of ammonia solution (even under the influence of sodium bicarbonate solution).

If this reaction is carried out with alkaline solutions, a result similar to the above can be obtained.

2.5.2. Reaction with aluminium chloride

A few drops of a 5% solution of aluminium chloride were added to a 5 ml solution of flavonoids in alcohol (or 5 ml alcohol extract of flavonoids prepared from plants) in a porcelain container, and a yellow colour was formed.

2.5.3. Reaction with lead acetate

We added lead (II)-acetate alcohol solution to the alcoholic solution of flavonoids in a porcelain container and mixed it. We found out that flavones, chalcones, and aurones with an ortho hydroxyl group in free form in lead (II)-acetate solution formed a clear yellow or red precipitate. When we used lead (II) hydro acetate solution instead of lead (II) acetate, all flavonoids gave a coloured precipitate. In this reaction,

anthocyanins formed a red or blue precipitate.

2.5.4. Reaction with mineral acids.

When hydrochloric acid was applied to the alcoholic solution of flavonoids in a porcelain container, all groups of flavonoids reacted with colour; flavones and flavonols were coloured clear yellow, and flavanones were coloured golden-pink, anthocyanins were coloured golden or red.

Chalcones and aurones have a red colour due to the formation of oxonium salts with a concentrated solution of acid.

In the case of taking concentrated sulfuric acid instead of hydrochloric acid, catechins, anthocyanins, and flavanones are coloured red, flavanes and flavonols are coloured from clear yellow to golden colour.

III. Results

At first, we picked the root, flower, and body parts of the blueberry plant for the experiment. We washed the collected topsoil with water to clean it from various soil bodies and others and stored it in a special place. For the laboratory work, we prepared the upper part of the plant by crushing it and conducted several qualitative reactions to the flavonoids contained in the plant.

1. Reaction with ammonia.

When we added ammonia solution to the solution of flavonoids in alcohol obtained in a porcelain container and heated it a little in a water bath, as a result of the reaction, the solution of flavones, flavonols, and flavonoids formed a yellow colour that changed to golden or red.

The addition of ammonia solution to the solution of chalcones and aurones produced a red or dark red colour without heating. Anthocyanins were coloured purple under the influence of ammonia solution (even under the influence of sodium bicarbonate solution).

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2. Reaction with aluminium chloride.

A few drops of a 5% solution of aluminium chloride were added to a 5 ml solution of flavonoids in alcohol (or 5 ml alcohol extract of flavonoids prepared from plants) in a porcelain container, and a yellow colour was formed.

3. Reaction with alkalis.

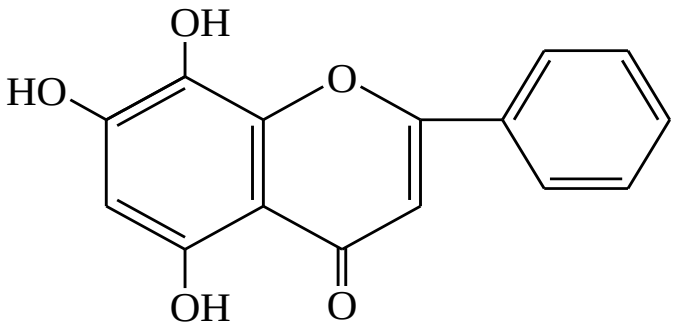
Most poly oxy flavonoid compounds, when dissolved in alkaline solutions, turned into coloured substances immediately or after a certain time.

The flavonoids became a colourless or faintly yellow solution when dissolved in cold dilute alkali. The isomerization process accelerated upon heating. In the case of flavonoids, in the case of free 4'-OH, the 7th position is substituted, and the effect of dilute alkalis on them was also very high. If the 4'-phenol group has been replaced, the chalcone change in isomerization does not produce bright colours. Chalcones formed red solutions when dissolved in dilute alkalis. Flavonols and flavanols often formed yellow solutions with alkalis.

4. Reaction with iron (III) chloride.

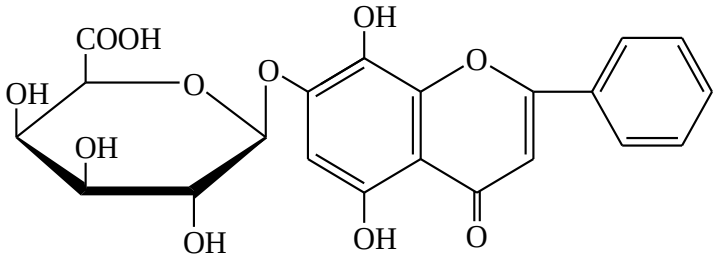
When we add a few drops of a 5% solution of iron (III) chlorides in alcohol to 5 ml solution of flavonoids in alcohol (or 5 ml alcohol extract of flavonoids prepared from plants) in a porcelain container, dark blue, dark purple, dark green or green colours were produced. When we compared it with the literature, we saw that the same colour was formed. We have seen that all groups of flavonoids have a colour reaction with iron (III) chloride solution. To extract flavonoids from the plant, we extracted the plant in alcohol and butanol and divided the obtained extracts into fractions. We diluted the alcohol obtained as a result of extraction with distilled water and extracted it with ethyl acetate in a separating funnel. As a result, we separated

the fraction with the ethyl acetate. When examined according to the results of the ethyl acetate sum, clear signs corresponding to the substance Norvogonin were observed.



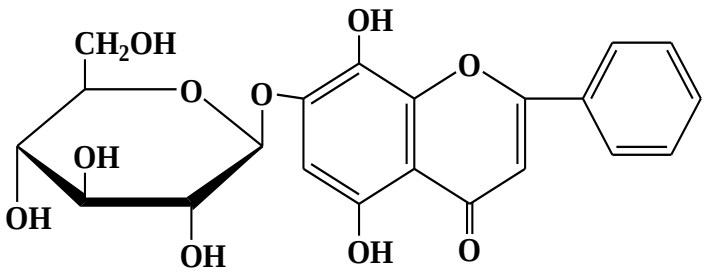
Norvogonin (5,7,8-trihydroxy flavone). General formula:$C_{15}H_{10}O_5$

1. In the thin layer chromatography of the sum with butanol, a yellow spot was observed in the system with the solvent chloroform-methanol (4:1), and in the system with the solvent chloroform-methanol (9:1), a light yellow spot was isolated. Chromatographic results were obtained by comparing the obtained substances with other similar substances.



Norvogonin 7—*O*—β—D- galacturonide - nepetoside A, (chloroform-methanol (4:1) General formula:$C_{21}H_{18}O_{11}$

The second substance was found to be Norvogonin 7—*O*—β—D-glucopyranoside



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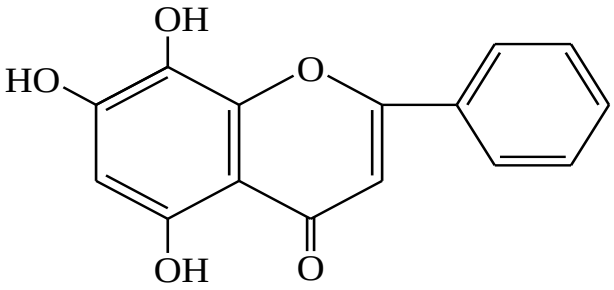

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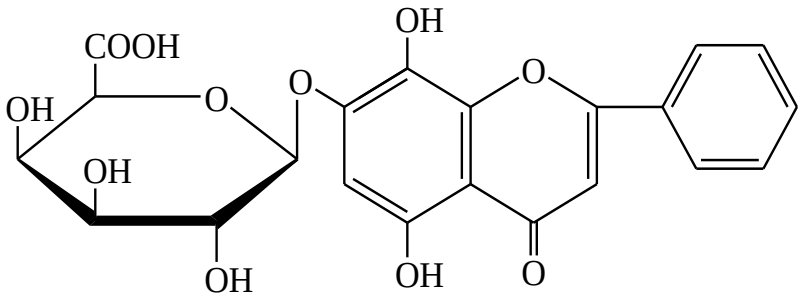

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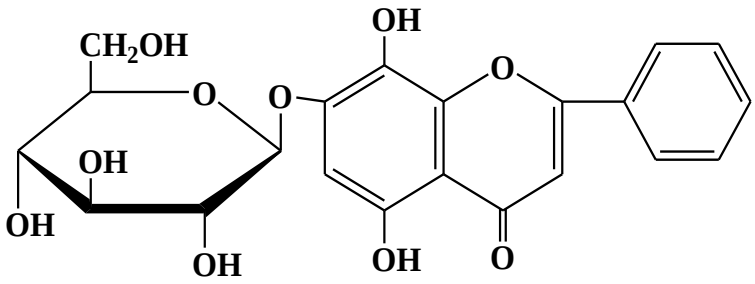

IV. Conclusion

1. Scutellaria leptosiphon Juz. practical works such as extraction, separation into fractions in solvents, paper chromatography, thin layer chromatography, and qualitative reactions were performed to extract flavonoids from the surface of the plant.

2. According to the results of the study of the composition of the ethylacetate sum, it was found that Norvogonin (5,7,8-trihydroxy flavone) is present.

3. According to the results of studying the composition of butanol sum

Norvogonin 7—*O*—β—D-glucopyranoside was found.

4. The substances obtained as a result of the experiments gave the results of the qualitative chromatographic analysis by the test substances, and it was the basis for our conclusion that their structure is the same as these substances.

5. We believe that the number of substances in the studied plant extracts, the adequacy of the raw material base, and the high pharmacological activity of this plant in the future, can be used in the field of pharmaceuticals.

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