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# Phytochemical analysis of *Petrosimonia sibirica* grown in Kazakhstan

**Abstract.** In this study, complete phytochemical analyses of the component composition of *Petrosimonia sibirica* was conducted for the first time. Phytochemical screening showed the presence of a variety of primary and secondary metabolites. The data for quantitative determination of biologically active compounds were presented. The qualitative composition of amino, fatty acids of the plant *Petrosimonia sibirica* has been studied by using the method of paper chromatography (PC) and thin-layer chromatography (TLC), their quantitative composition of amino, fatty acids have been identified by gas chromatography. The composition of 20 amino, 8 fatty acids of *Petrosimonia sibirica*, family *Chenopodiaceae* have been established. The major amino acids in studied plant were glutamic acid (2.440%), alanine (0.618%), aspartic acid (1.254%), arginine (0.405%), tyrosine (0.340%), and proline (0.306%). The dominant fatty acids in plant with respect to quantity were oleic (48.3%) and linoleic (26.7%) acids. In order to optimize the extraction process technological parameters (extracting solvent, a ratio between solvent and solid plant material and process temperature) were selected. The most suitable solvent is 70% ethyl alcohol, solid – solvent ratio (1: 6-8), extraction time (3 days), and extraction temperature (20-25 °C). In addition, Kazakh species of *Petrosimonia sibirica* plant are valued as a rich source of saponins and flavonoids. Quercetin 3-O-β-D-glucopyranoside (isoquercitrin) was isolated from *Petrosimonia sibirica*. Flavonoid glycoside was identified by $^{1}$H NMR, $^{13}$C NMR, and MS, and compared with the data in the literature. The results revealed the presence of medicinally important constituents in the studied plant. Therefore, extracts from these plants could be seen as a good source for useful drugs.
**Key words:** *Petrosimonia sibirica*, halophytes, amino acids, fatty acids, flavonoids, quercetin 3-O-β-D-glucopyranoside (isoquercitrin).

## Introduction

Despite the intensive growth of medicinal products, plants continue to occupy a significant place in the arsenal of medicines, since their lesser side effect, accumulation in the body, as well as the effectiveness in the treatment of certain diseases, have been proved.

The importance of plants is known to us well. The flora of the Republic of Kazakhstan has the richest reserves of plant resources, but it is not used enough in medical practice, which requires more in-depth study and introduction into the domestic medicine of effective medicines derived from plant materials.

Plant cells produce two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids, and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins, and tannins, etc.). They act as defense chemicals [1]. Secondary metabolites are also relevant to medicine and agriculture.

Therefore, the study of the chemical composition of the plant materials, development of methods for the isolation of biologically active substances and the study of biological activity in order to isolate new drugs and herbal remedies is an important task.

Plants of the family *Chenopodiaceae*, which occupy the predominant part of the landscape of the Republic of Kazakhstan, are of great interest. Chemical studies of most plants of this family indicate their high nutritional value. From the literature, it is known that plants growing in arid zones have

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an interesting chemical composition and practical significance.

*Petrosimonia sibirica* (Pall.) Bunge belongs to the family *Chenopodiaceae*, a family comprising of probably about 100 genera and 1400 species, which is represented in Kazakhstan by 47 genera and 218 species. There are 11 species of the plant genus *Petrosimonia*; 10 of these are indigenous to Kazakhstan. *Petrosimonia* is the basic fodder for camels in the autumn [2, 3]. According to literary studies, phenolic compounds, an alkaloid, quinone, lactone, and esters have been isolated from plant *Petrosimonia sibirica*, which grown in China [4, 5]. However, there are no reports on phytochemical analysis of Kazakh species of the plant genus *Petrosimonia*.

The investigation of the chemical constituents from the aerial part of *Petrosimonia sibirica* was reported for the first time.

## Materials and methods

Melting points were determined in open capillary tubes on Buchi M-560 melting point apparatus and are uncorrected. NMR spectra were recorded using $CD_3OD$ as solvent on Avance AV 400 MHz Instrument (Bruker Co., Switzerland). Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. The $^{1}H$, $^{13}C$-NMR and ESI-MS spectra were recorded at HEJ Research Institute of Chemistry, University of Karachi, Pakistan. Thin layer chromatography (TLC) was performed by using silica gel plates Alugram SIL G/UV 254 (Macherey-Nagel, Germany) and identification of the spots on the TLC plate was carried out by spraying *ceric sulfate*.

*Plant material.* The aerial part of *Petrosimonia sibirica* was collected during flowering period from saline soils at Karatal district of Almaty region, Kazakhstan. The plant material was taxonomically identified, authenticated by professors of botany at Institute of Botany and Phytointroduction, Almaty. The aerial parts of the plant were air dried, powdered to particle size in the range 6.0-8.0 mm, according to regulatory documents, sieved, weighed and transferred into airtight containers with proper labeling for future use.

*Extraction and Isolation.* The air-dried and finely powdered aerial parts of the plant (1.0 kg) wad exhaustively extracted by maceration for 72 hrs. at room temperature with ethanol (70%) till complete exhaustion. Extraction is repeated twice. The ethanolic extract was concentrated under reduced pressure using rotary vacuum evaporator to obtain a dark brown residue (130 g). The combined extract was dissolved in a least amount distilled water/alcohol mixture (9:1) and successively extracted with hexane, chloroform, ethyl acetate, and *n*-butanol. Each extract was separately evaporated to dryness using rotary vacuum evaporator under reduced pressure at a temperature not exceeding 45 ºC. Each of the obtained fractions was subjected to thin layer (TLC) and paper (PC) chromatographic techniques and then column chromatography for isolation of its major constituents. The ethyl acetate soluble extract of *Petrosimonia sibirica* (28 g) was chromatographed on silica gel column using different ratio of chloroform and methanol in gradient elution manner followed by Sephadex LH-20 with MeOH to yield 17 mg of compound 1.

*Acid hydrolysis* of the compound carried by 2% HCl on a boiling water bath, while mild acid hydrolysis was sampled at a certain time interval. The hydrolysis products were identified by m.p., PC, and TLC in appropriate solvent systems with authentic samples.

*Fatty acids analysis.* The composition of the saturated and unsaturated carboxylic acids (fatty acids) in plants is determined by gas-liquid chromatography apparatus Carlo-Erba-4200 using helium as a carrier gas, flame ionization detector, carrier gas velocity 30 ml/min, detector temperature 188 ºC, oven temperature 230 ºC, adsorbent Cellite 545 on Chromosorb WAW. The chloroform extract of plant species is added to 10 ml of methanol and 2-3 drops of acetyl chloride and then carried out methylation at 60-70 ºC in a special system for 30 minutes. Methanol was removed using a rotary evaporator, and the samples are extracted with 5 mL of *n*-hexane and analyzed by gas chromatography for 1 hour [6].

*Amino acids analysis.* Analysis of amino acids was carried out chromatographically using helium as carrier gas, flame ionization detector 300 ºC and condenser temperature 250 ºC on Chromosorb WAW. Aqueous extract of the plant was hydrolyzed in HCl for 24 hours. The resulting hydrolyzate was evaporated to dryness in a rotary evaporator at 40 ºC, after centrifugation at 2.5 thousand revolutions per minute the resulting precipitate was dissolved in sulfosalicylic acid and amino acids are eluted through an ion exchange column Dausk-50. On freshly obtained elutes 2, 2-dimethoxypropane and propanol saturated with HCl were added. The resulting mixture is heated at 110 ºC for 20 minutes, then addition of a freshly prepared acylating reagent (1 volume of acetic anhydride and 2 volumes of triethylamine and 5 volumes of acetone), evaporation of the sample to dryness, addition of ethyl acetate and saturated aqueous solution

of NaCl. Finally, the ethyl acetate layer is analyzed on the amino acid analyzer (Carlo-Erba) [7].

*Vitamin analysis.* The contents of vitamins A (retinol) and E (tocopherol) were determined by fluorimetry on a spectrofluorometer (Hitachi, Japan). The vitamin C content in the biological samples was determined by titration [8-10]. Fatty and amino acids, vitamin analyses were performed at the laboratories of The Kazakh Academy of Nutrition.

## Results and discussion

The moisture content, total ash, extractives, qualitative and quantitative contents of biologically active constituents of *Petrosimonia sibirica* were determined according to methods reported in the State Pharmacopoeia of the Republic of Kazakhstan I edition techniques [11].

Moisture content is an important factor because the appearance and stability of dried plants depend on the amount of water they contain and the propensity of microorganisms to grow depends on their water content. Medicinal plant materials should not contain moisture above the permissible norms; since at high humidity, during storage conditions are created that contribute to a decrease in its quality. For most types of medicinal plant materials, the permissible limit of moisture is usually 12–15%. The results in table 1 showed low moisture content of the aerial part in *Petrosimonia sibirica* (7.8%).

The amount and composition of ash remaining after combustion of plant material vary considerably according to the part of the plant, age, environment etc. The ash content is a measure of the total amount of minerals present within a plant, whereas the mineral contents are a measure of the amount of specific inorganic components present within it [12].

The extractives of medicinal plants conventionally called complex organic and inorganic substances extracted from plant material with an appropriate solvent and quantified as a dry residue.

The methods of two- and one-dimensional chromatography on paper, as well as TLC in various solvent systems established for the first time that the following main groups of biologically active substances are contained in the studied plant: flavonoids, amino acids, alkaloids, saponins, coumarins, and carbohydrates.

Vitamins are defined as relatively low-molecular-weight compounds which humans, and for that matter, any living organism that depends on organic matter as a source of nutrients, require small quantities for normal metabolism. There are more than 30 such substances, and all of them are vital for the human body, entering into the composition of all tissues and cells, activating and determining the course of many processes. Vitamins increase the body's resistance to infectious diseases, inhibit the aging process, determine the activity of enzymes, participate in the metabolism of amino acids, fatty acids, hormones, microelements [10]. Vitamins are classified as either fat-soluble (vitamins A, D, E and K) or water-soluble (vitamins B and C). The results indicated that vitamin C dominated over vitamins E and A (table 1).

The data quantitative determination of *Petrosimonia sibirica* is shown in Table 1. The results revealed the presence of biologically active compounds in the plant studied.

From table 1, it could be seen the predominance of saponins and flavonoids in *Petrosimonia sibirica*.

**Table 1** – Qualitative and quantitative screening of the powdered aerial parts of *Petrosimonia sibirica*

| Plant | Contents, % | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moisture content | Ash | Extractives materials 70% – aqueous alcohol | Saponins | Flavonoids | Tannins | Alkaloids | Carbohydrates | Organic acids | Coumarins | Vitamin A | Vitamins C | Vitamins E |
| *Petrosimonia sibirica* | 7.8 | 24.7 | 52.9 | 0.6 | 2.0 | 0.1 | 0.4 | 4.2 | 3.5 | 0.3 | 0,00016 | 0,011 | 0,0022 |

It is known that amino acids occupy a special place in modern medicine. By their action, many of them belong to central neurotransmitters, both stimulating and inhibiting the transmission of nerve impulses in the synapses of the central nervous system, which determines their pharmacological orientation. In total, about 300 amino acids have been found in nature, however, only 20, which are called protein or proteinogenic amino acids, are found in proteins. Having a wide range of pharmacological actions and the ability to enhance the digestibility of other substances, amino acids are attracting more and more attention of researchers as potential drugs. One of the most important functions of amino acids is their participation in the synthesis of proteins that perform catalytic, regulatory, spare, structural, transport, protective and other functions [13, 14].

GC analysis of the amino acids constituents of the aerial part of *Petrosimonia sibirica* (table 2) revealed the presence of twenty amino acids but differs in their percentages; the major amino acids in studied plant were glutamic acid (2.440%), alanine (0.618%), aspartic acid (1.254%), arginine (0.405%), tyrosine (0.340%), and proline (0.306%).

*Petrosimonia* plants can be used in autumn and winter as wild feed for sheep and cattle owing to the high contents of glutamic and aspartic acids.

**Table 2** – Amino acids composition of *Petrosimonia sibirica*, %

| Amino acids | Relative percentage % | Amino acids | Relative percentage % |
|---|---|---|---|
| Alanine | 0.618 | Cysteine | 0.032 |
| Glycine | 0.296 | Oxyproline | 0.001 |
| Valine | 0.274 | Phenyl alanine | 0.290 |
| Leucine | 0.380 | Glutamic acid | 2.440 |
| Isoleucine | 0.362 | Ornithine | 0.001 |
| Threonine | 0.202 | Tyrosine | 0.340 |
| Serine | 0.204 | Histidine | 0.260 |
| Proline | 0.306 | Arginine | 0.405 |
| Methionine | 0.060 | Lysine | 0.202 |
| Aspartic acid | 1.254 | Tryptophan | 0.094 |

Probably, the appearance of fatty acids in plant extract is associated with the hydrolysis of lipids in plants. Fatty acid glycerides are physiologically active, especially glycerides of some unsaturated fatty acids. These include linoleic and linolenic acids, which are necessary for the vital activity of a living organism (vitamin F factor) [15, 16].

Fatty-acid analyses for studied plant detected eight fatty acids (table 3). The dominant fatty acids in studied plant with respect to quantity were oleic (48.3%) and linoleic (26.7%) acids. This fact and the rapidly renewable properties together with high drought and freezing resistance and broad distributions on low and highly saline soils, i.e., those of little value for agriculture, supported our hypothesis about the feed value of *Petrosimonia* plants.

In order to optimize the extraction process of biologically active compounds from *Petrosimonia sibirica* technological parameters were selected. Extracting solvent, a ratio between solvent and solid plant material and process temperature are the most important optimum extraction parameters. A quantitative measure for this is the valuable compounds extraction yield from plant material. The most suitable solvent is 70% ethyl alcohol, solid – solvent ratio (1: 6-8), extraction time (3 days), and extraction temperature (20-25 $^{o}$C), under these conditions, up to 60% of biologically active compounds are extracted.

Powdered plant material of *Petrosimonia sibirica* was soaked in 70% ethanol, and then ethanolic extract was concentrated. It was then divided into *n*-hexane, chloroform, ethyl acetate, *n*-butanol, and aqueous fractions.

The *n*-hexane fraction contains large amounts of chlorophyll which hindered the isolation of its contents, together with fatty acids, sterols, and resins. The chloroform fraction has shown the presence of several secondary metabolites such as coumarins, alkaloids along with fat-soluble vitamins. The ethyl

acetate fraction was found rich in polyphenolics and terpenoids. Saponins were found in the *n*-butanol fraction.

**Table 3 –** Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Petrosimonia sibirica*, %

| Fatty acids | Content, % |
|---|---|
| Linoleic acid [$C_{18:2}$] | 26.7 |
| Oleic acid [$C_{18:1}$] | 48.3 |
| Palmitic acid [$C_{16:0}$] | 14.7 |
| Stearin acid [$C_{18:0}$] | 5.2 |
| Palmitoleic acid [$C_{16:1}$] | 0.9 |
| Pentadecanoic acid [$C_{15:0}$] | 1.6 |
| Myristic acid [$C_{14:0}$] | 1.3 |
| Linolenic acid [$C_{18:3}$] | 1.3 |

Since the purpose of this work is to isolate flavonoids, ethyl acetate soluble fraction has been studied more deeply. The $^{1}$H NMR, $^{13}$C NMR spectrum of compound 1 revealed the characteristic signals of flavonol glycosides. Compound 1 was identified by ESI-MS, which gave molecular ion peak at *m/z* 464, corresponding to the molecular formula $C_{21}H_{20}O_{12}$.

Compound 1 – yellow crystals, m.p. 230-232 ºC, have a dark glow in UV light. IR spectrum (KBr, $\nu_{max}$, $cm^{-1}$): 3350-3256, 1645, 1115-1061. UV spectrum (MeOH, $\gamma_{max}$, nm): 362, 264. The position on the two-dimensional paper chromatogram (*n*-butanol–acetic acid–water (BAW), 40:12.5:29, 6 % acetic acid) of compound indicates their glycosidic nature [17]. From the products of acid hydrolysis of compound 1, the corresponding aglycone was isolated, in the hydrolysate by the PC method using o-toluidine as a developer, glucose were identified in comparison with reliable samples.

In the products of alkaline destruction of the aglycone of compound 1, it has been found that rings A have the structure of phloroglucinol, i.e. in the C-5 and C-7 positions, there are free hydroxyl groups, and ring B of compound 1 is defined as protocatechuic acid [18].

$^{1}$H NMR (500 MHz, CD3OD, $\delta$, ppm): 7.68 (1H, d, J=2.0, H-2′), 7.61 (1H, d, H-6′), 7.30 (1H, d, J=8.0, H-5′), 6.64 (1H, d, J=2.0 Гц, H-8), 6.45 (1H, d, J=2.0, H-6), 5.54 (d, J=8.0, H-1′′).

From the data of 1H NMR spectroscopy, it follows that carbohydrate residue in compound 1 is in the β-form. To determine the place of addition of sugar, $^{13}$C-NMR and HMBC two-dimensional spectra were taken. Based on modern spectral analysis methods and comparison with the literature data, accordingly, compound 1 was proved to be quercetin 3-O-β-D-glucopyranoside (isoquercitrin) [19, 20].

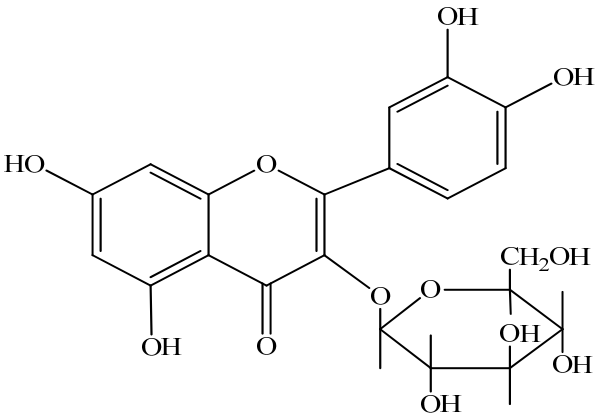


**Figure 1** – Quercetin 3-O-β-D-glucopyranoside (Compound 1)

## Conclusion

A phytochemical study of *Petrosimonia sibirica* was carried out. The qualitative composition of amino, fatty acids of the plant *Petrosimonia sibirica* has been studied by using the method of paper chromatography (PC) and thin-layer chromatography (TLC), their quantitative composition of amino, fatty acids have been identified by gas chromatography.

Column chromatography of ethyl acetate soluble fraction obtained from the ethanolic extract afforded quercetin 3-O-β-D-glucopyranoside (isoquercitrin), which was identified by comparison of its NMR with reported data. Flavonoid glycoside was isolated from Petrosimonia sibirica for the first time.

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## References

1. Irchhaiya R., Kumar A., Yadav A., Gupta N., Kumar S., Gupta N., et al. (2015) Metabolites in plants and its classification. *World J. Pharm. Pharmaceut. Sci*, vol. 4, pp. 287-305.

2. Pavlova N.V. (1960) Flora of Kazakhstan [Flora Kazakhstana], *Akad. Nauk KazSSR*, Alma-Ata; vol. 3, pp. 306-311 (in Russian)

3. Komarov V.L. (1955) Flora of the USSR [Flora SSSR], *Akad. Nauk SSSR*, Moscow, Leningrad; vol. 6, pp. 316-326 (in Russian)

4. Sun W., Ma Z.Y., Zhang X., Yang H.B., Sun W.F. (2015) Secondary metabolites of *Petrosimonia sibirica*. *Chem. Nat. Compd.*, vol. 51, pp. 530.

5. Wang Y., Zhang H., Su X., Yang H.B., Sun W.F. (2016) Flavonoids and phenolic compounds of *Petrosimonia sibirica*. *Chem. Nat. Compd.*, vol. 52, pp. 482.

6. Chamberlain J., Nelson G., Milton K. (1993) Fatty acid profiles of major food sources of howler monkeys (*Aloutta palliata*) in the neotropics. *Experientia*, vol. 49, pp. 820-823.

7. Adams R.F. (1974) Determination of amino acid profiles in biological samples by gas chromatography. *Journal of Chromatography A*, vol. 95 (2), pp. 189-212.

8. Neeld J.B., Pearson W.N. (1963) Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. *The Journal of Nutrition*, vol. 79(4), pp. 454-462.

9. Omaye S.T., David Turnbull J., Sauberlich H.E. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. *Vitamins and coenzymes*, Part D, pp. 3-11.

10. Pegg R.B., Landen W.O., Eitenmiller R.R. (2010) *Vitamin Analysis. Food Analysis*, pp. 179-200.

11. State Pharmacopoeia of the Republic of Kazakhstan [Gosudarstvennaia farmakopeia Respubliki Kazakhstan], (2008) *Zhibek Joly*, Almaty (in Russian)

12. Seitimova G.A., Alzhanbayeva A.M., Burasheva G.Sh., Yeskaliyeva B.K., Choudhary M.I. (2016) Phytochemical study of *Kochia prostrata. International Journal of Biology and Chemistry*, vol. 9 (2), pp. 51-54.

13. McKee T., McKee J.R. (2012) Biochemistry: The molecular basis of life, 5th edition. *Oxford University Press*, Oxford, New York.

14. Tiukavkina N.A., Baukov Yu.A. (2004) Biiorganic chemistry [Bioorganicheskaia khimiia]. *Drofa*, Moscow (in Russian)

15. Watson R.R., Fabien De Meester (2016) Handbook of lipids in human function: Fatty acids. *Academic Press and AOCS Press*, USA, 809 p.

16. Kurkin V.A. (2004) *Pharmacognosy: Textbook for Students of Pharmaceutical Higher Educational Institutes* [Farmakognoziia]. *OOO Ofort*, Samara, pp. 141-186 (in Russian)

17. Mabry T.I., Markham K.R., Thomas M.B. (1970) The Systematic Identification of Flavonoids. *Springer-Verlag,* Berlin-Heidelberg-New-York, pp. 3-14.

18. Burasheva G.S., Mukhamed'yarova M.M., Chumbalov T.K. (1976) Polyphenols of *Alhagi kirgisorum.* III, *Chem. Nat. Compd.*, vol. 12(5), pp. 596.

19. Markham K.R. (1982) Techniques of flavonoid identification. *Academic Press,* London-New York, 113 p.

20. Kim H.Y., Moon B.H., Lee H.J., Choi D.H. (2004) Flavonol glycosides from the leaves of *Eucommia ulmoides* O. with glycation inhibitory activity. *Journal of Ethnopharmacology*, vol. 93(2-3), pp. 227-230.