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Investigation of chemical constituents of medicinal Plant *Spiraea Hypericifolia* L.

Abstract: In this work, the quantitative analysis of phytochemical constituents of medicinal plant *Spiraea hypericifolia* L. from Kazakhstan has been made for the first time. Total bioactive components aerial and underground parts of plants material *S. hypericifolia* L. such as organic acids (0.28 %), (0.35 %), flavonoids (0.77 %), (5.36 %), coumarins (0.04 %), (0.21 %), saponins (2.09 %), (0.98 %), tannins (1.31 %), (1.62 %), and together with moisture content (4.72 %), (3.84 %), total ash (5.11 %), (5.41 %), were determined. Eleven macro-micro elements from the ash of plant were identified, main contents of them were Ca (362.20 μg/ml), (344.750 μg/ml), K (69.670 μg/ml), (57.8075 μg/ml), and Mg (24.270 μg/ml), (34.480 μg/ml), by using the method of multi-element atomic emission spectral analysis. In addition, twenty amino and eight fatty acids were analyzed from the plant. The results showed that major contents of amino acids were glutamate (1741 mg/100g), (1710 mg/100g), aspartate (845 mg/100g), (820 mg/100g), and alanine (560 mg/100g), (523 mg/100g), as well as in fatty acid was linoleic (81.1 %), (79.7 %) acid, respectively.
Key words: *Spiraea hypericifolia* L., bioactive constituents, macro-micro elements, amino-, fatty acids.

Introduction

Studies on phytochemicals have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand [1]. The genus *Spiraea* L., spirea, represents deciduous shrubs of the family *Rosaceae Juss*., subfamily *Spiraeoideae* Focke, respectively the family *Spiraeaceae* Humb., Bonpl. & Kunth in the narrower concept. The genus is widespread in the temperate and the subtropical zone of the northern hemisphere having more than 100 species [2-3]. *S. hypericifolia* L. has the most extensive Eurasian range and is considered one of the most evolutionarily advanced representatives of the genus [4]. In Europe, beside two native species, *S. hypericifolia* L. and *S. salicifolia* L., cultivated evidently already in the 16th or 17th century, the first species being imported from overseas were East American *S. tomentosa* L. and *S. alba* Du Roi. A species with an extensive natural geographic range in Eurasia. The typical subspecies is distributed from East Europe to Central Asia, West Mongolia, North China and SouthEast Siberia (Transbaikalia), while the subsp. *obovata* occurs in SouthWest Europe [5]. Deciduous shrub 0, 5-1, 6 m height. Crohn thick, sprawling. Branches are numerous, spreading or arcuate, brown. Very thin, curved, angular, red-brown, first pubescent, then naked[6]. In the leaves of *S. hypericifolia* L. detected, p-hydroxybenzoic, coffee, ferulic, chlorogenic acid, flavones apigenin, luteolin and 5-glucosides of flavonols isoquercitrin and avicularin [7]. In the hydrolysates of *S. hypericifolia* L. discovered quercetin, chlorogenic, p- coumaric and caffeic acids in extracts – hyperoside, isoquercitrin, avicularin[8].

This study has made the investigation of the chemical constituents from Kazakh medicinal plants of *S. hypericifolia* L. grown in Almaty region of Kazakhstan for the first time.

Materials and methods

Plant material. The aerial and underground parts of plants material *S. hypericifolia* L. was collected in Almaty region Kazakhstan in October, 2018. The air dried aerial and underground parts of *S. hypericifolia* L. was cutted into small pieces and stored at room temperature.

Experimental part. The quantitative determination of detected groups of natural compounds is carried out according to the method of the State Pharmacopoeia and the methodology developed by the author of phytochemical analysis [9-12]

In the "Center of Physical and Chemical methods of research and analysis", Republican State Enterprise Kazakh National Al-Farabi University, Ministry of Education and Science of RoK using the method of multi-element atomic emission spectral analysis in the ash of *S. hypericifolia* L. were analyzed elemental constituents. To determine the mineral composition of ashes was used Shimadzu 6200 series spectrometer.

Method for the determination of amino acids. 1 g of the analyte, hydrolyzed in 5 ml of 6N hydrochloric acid at 105 ºC for 24 hours, in ampoules sealed under a stream of argon. The resulting hydrolyzate is evaporated three times to dryness on a rotary evaporator at a temperature of 40-50 ºC and a pressure of 1 atm. The resulting precipitate is dissolved in 5 ml of sulfosalicylic acid. After centrifugation for 5 minutes, the packed liquid is passed through a column of ion exchange resin at a rate of 1 drop per second. After this, the resin is washed with 1-2 ml of deionized water and 2 ml of 0.5N acetic acid; then the resin is washed to neutral pH with deionized water. To elute the amino acids from the column, 3 ml of a 6N NH_4OH solution is passed through it at a rate of 2 drops per second. The eluate is collected in a round bottom flask together with distilled water, which is used to wash the column to a neutral pH medium. The contents of the flask are then evaporated to dryness on a rotary evaporator at a pressure of 1 atm and a temperature of 40-50 ºC. After adding a drop of freshly prepared 1.5% $SnCl_2$ solution, 1 drop of 2,2-dimethoxypropane and 1-2 ml of propanol saturated with hydrochloric acid, it is heated to 110 ºC, keeping this temperature for 20 minutes, and then the contents are again evaporated from the flask on a rotary evaporator. In the next step, 1 ml of freshly prepared acetyl reagent (1 volume of acetic anhydride, 2 volumes of triethylamine, 5 volumes of acetone) is introduced into the flask and heated at a temperature of 60 ºC for 1.5-2 minutes. The sample is again evaporated on a rotary evaporator to dryness and 2 ml of ethyl acetate and 1 ml of a saturated NaCl solution are added to the flask. The contents of the flask are thoroughly mixed and as the two layers of liquids are clearly formed, an upper layer (ethyl acetate) is taken for gas chromatographic analysis.

To determine the amino acids composition was made erenow [13] of the raw material used GC/MS device. GC/MS analysis: the aerial and underground parts of *S. hypericifolia* L. were analyzed by Gas Chromatograph coupled to Mass Spectrometer using polar mixture of 0.31% carbowax 20 m, 0.28% silar 5 CP and 0.06% lexan in chromosorb WA-W-120-140 mesh., column (400 x 3 mm). The column temperature was programmed from 110ºC (held for 20 min), at 6ºC/min from 110ºC to 180ºC, at 32ºC/min from 185ºC to 290ºC. When it reaches to 250ºC, it should stay constant till finishing analysis of all existed amino acids. The chromatogram is counted according to an external standard.

Determination of the fatty acids composition of dried plants aerial and underground parts of *S. hypericifolia* L. extracted with a chloroform- methanol mixture (2:1) for 5 minutes, the extract is filtered through a paper filter and concentrated to dryness. Then, to taked extract add 10 ml of methanol and 2-3 drops of acetyl chloride and further methylation at 60-70°C in a special system for 30 minutes. The methanol is removed by rotary evaporation and the samples are extracted with 5 ml of hexane and analyzed using a gas chromatograph together with MS by applying the same method in investigation of amino acids.

As a result, chromatograms of methyl esters of fatty acids were obtained. By comparison with reliable samples by the time of exit from the column, eight fatty acids were identified. To determine the components was used the internal normalization method.

Results and discussion

The quantitative analysis of biologically active constituents together with moisture content, total ash were determined from aerial and underground parts of *S. hypericifolia* L. The results are shown in Table 1.

Moisture and ash contents depend on many factors, such as the method of collection and the method of drying. These indicators have a certain limit. For example, for my plant limit is 12%. Based on the results we can say that the goodness of the plant *S. hypericifolia* L has been proven. This plant might be used in the pharmaceutical industry.

As values were determine with a purpose to find out the total amount of inorganic solutes present in the medicinal plant material. Quite a few herbal therapies make use of ash. It is very obvious that ash of any plant does not contain any organic material and therefore inorganic salts are used medicinally. It is also interesting to know about the different solubility of the components of ash. Therefore, the solubility of ash in water and hydrochloric acid was tested in the present study. Organic acids play an important role in maintaining the acid-base balance of the human body. Organic acids are responsible for the taste, the flavour, the microbial stability, and the product consistence of plant derived beverages and are used in food preservation because of their effects on bacteria. Flavonoids are a class of compounds presented broadly in nature. Concerns about their extensive profitable bioactive benefits, including anti-viral/bacterial, anti-inflammatory, cardio protective, anti-diabetic, anti-cancer, anti-aging, have long been received great attention and well supported by numerous studies. Mostly, phytochemicals from the group of flavonoids have been reported as the major contributor to the biological activities. It is believed that the significant biological activities exhibited by the herbal materials are due to the presence of flavonoids acting as antioxidants.

Table 1 – Quantitative analysis of bioactive constituents of *S. hypericifolia* L.

Content, %							
Plants	Moisture content	Ash	Organic acids	Flavanoids	Coumarins	Saponins	Tannins
Aerial part of S. hypericifolia L.	4.72	5.11	0.28	0.77	0.04	2.09	1.31
Underground part of S. hypericifolia L.	3.84	5.41	0.35	5.36	0.21	0.98	1.62

Macro-micro elemental composition

In "Center of Physical and Chemical methods of research and analysis", Republican State Enterprise Kazakh National Al-Farabi University, Ministry of Education and Science of RoK using the method of multi-element atomic emission spectral analysis in the ash of *S. hypericifolia* L. there were determined eleven macro- and microelements, shown in Table 2 and Figure 1,2 and major of them was Ca (362.20 μg/ml), (344.750 μg/ml), K (69.670 μg/ml), (57.8075 μg/ml) and Mg (24.270 μg/ml), (34.480 μg/ml). The information helps to select plants with high quantity of each nutrient. This will intern confirm the efficacy of the medicinal activity of the plant. An analysis of macro and microelements showed a large variability. The present study showed a large variation in the contents of nutrients and protein %, thereby offering opportunity by scientists working on medicinal. 2 medicinal plants were grouped based on the maximum content of macro and micro elements. Potassium is an electrolyte, a substance that conducts electricity in the body. K is crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function[14]. The role of K in the human body is complex intervening in the forming proteins, maintaining cellular balance, acid-base balance, transport of oxygen and carbon dioxide in the blood, nerve impulse management, muscle contraction in cardiac muscle specuila, glycogenesis[15]. Magnesium is involved in hundreds of enzyme reactions in the body as it performs an array of biological functions as activation of muscles and nerves, digestion of proteins, carbohydrates, fats, building block for RNA and DNA synthesis. In the human body calcium, helps alongside phosphorus, the formation and strengthening of bones and teeth. Performs a number of functions among which the muscular contraction, nerve impulse transmission, immunity, promotes the absorption of iron and vitamin B12 is a blood clotting activator of various enzymes[16].

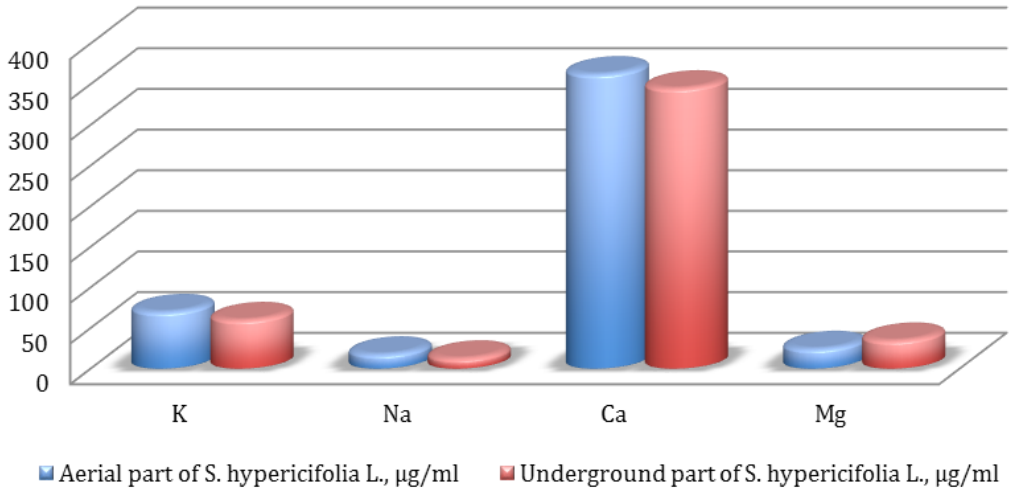

Figure 1 – Contents of macro elements of plants *S. hypericifolia* L.

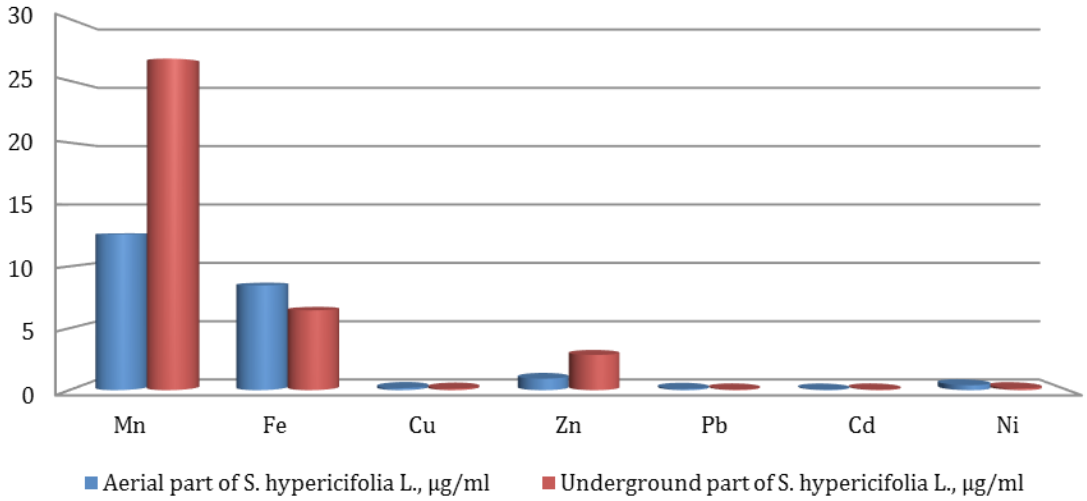

Figure 2 – Contents of micro elements of plants *S. hypericifolia* L.

Table 2 – Composition of macro-micro elements in the ash of plants *S. hypericifolia* L.

Element, µg/ml	K	Na	Ca	Mg	Mn	Fe	Cu	Zn	Pb	Cd	Ni
Aerial part	69.670	17.487	362.200	24.270	12.595	8.468	0.169	0.927	0.105	0.045	0.402
Under-ground part	59.807	12.542	344.750	34.480	26.785	6.448	0.122	2.846	0.055	0.055	0.156

Amino acid composition

Thus, in the quantitative determination of the amino acid composition of the aerial and underground parts of S. hypericifolia by the method of gas-liquid chromatography, 20 amino acids were detected. Their main content is represented by glutamate (1741 mg/100g), (1710 mg/100g), aspartate (845 mg/100g), (820 mg/100g) and alanine (560 mg/100g), (523 mg/100g). A smaller amount contains ornithine and oxyproline. The results shown in Table 3.

Table 3 – Amino acids contents of *S. hypericifolia* L.

№	Amino acids	Molecular formula	Amount in aerial part, mg/100g	Amount in underground part, mg/100g
1	Alanine	$C_3H_7NO_2$	560	523
2	Glycine	$C_2H_5NO_2$	180	161
3	Leucine	$C_6H_{13}NO_2$	320	295
4	Isoleucine	$C_6H_{13}NO_2$	294	270
5	Valine	$C_5H_{11}NO_2$	230	211
6	Glutamate	$C_5H_9NO_4$	1741	1710
7	Threonine	$C_4H_9NO_3$	218	202
8	Proline	$C_5H_9NO_2$	302	180
9	Methionine	$C_5H_{11}NO_2S$	50	41
10	Serine	$C_3H_7NO_3$	188	134
11	Aspartate	$C_4H_7NO_4$	845	820
12	Cysteine	$C_3H_7NO_2S$	29	22
13	Oxyproline	$C_5H_9NO_3$	1	2
14	Phenylalanine	$C_9H_{11}NO_2$	278	256
15	Tyrosine	$C_9H_{11}NO_3$	300	82
16	Histidine	$C_6H_9N_3O_2$	236	218
17	Ornithine	$C_5H_{12}N_2O_2$	1	2
18	Arginine	$C_6H_{14}N_4O_2$	328	305
19	Lysine	$C_6H_{14}N_2O_2$	212	190
20	Tryptophan	$C_{11}H_{12}N_2O_2$	66	53

Therefore, according to the results, *S. hypericifolia* L. could be one of the most valuable sources of these amino acids and might be widely used in medicine due to the fact that glutamate is one of the most abundant of the amino acids. In addition to its role in protein structure, it plays critical roles in nutrition, metabolism and signaling. Post-translational carboxylation of glutamyl residues increases their affinity for calcium and plays a major role in hemostasis [17]. Aspartic acid increases immunity, metabolism, deactivates ammonia, participates in the formation of ribonucleic acids, promotes the removal of chemicals, including drugs, restores working capacity. Studies conducted by scientists have proved the effectiveness of taking asparaginic acid preparations for increasing testosterone levels. Aspartic acid is taken as an additive by bodybuilding athletes to improve strength, increase libido and testosterone in the blood [18]. Alanine also increases immunity and provides energy for brain and central nervous system, the muscle tissue. This amino acid protects against the development of cancer of the pancreas and prostate gland [19].

Fatty acids composition

The result of gas-liquid chromatography determined the amount of 8 fatty acids. Quantitative composition of fatty acids in the aerial and underground parts of *S. hypericifolia* L. mostly contained in linoleic acid (81.1 %), (79.7 %), showed in Table 4.

Table 4 – Fatty acids contents of *S. hypericifolia* L.

№	Fatty acids	Molecular formula	Amount in aerial part, %	Amount in underground part, %
1	Myristic acid $C_{14:0}$	$C_{14}H_{28}O_2$	2.3	2.2
2	Pentadecanoic acid $C_{15:0}$	$C_{15}H_{30}O_2$	2.4	2.0
3	Palmitic acid $C_{16:0}$	$C_{16}H_{32}O_2$	5.2	4.9
4	Palmitoleic acid $C_{16:1}$	$C_{16}H_{30}O_2$	0.9	0.8
5	Stearin acid $C_{18:0}$	$C_{18}H_{36}O_2$	3.0	2.8
6	Oleic acid $C_{18:1}$	$C_{18}H_{34}O_2$	6.3	6.1
7	Linoleic acid $C_{18:2}$	$C_{18}H_{32}O_2$	81.1	79.7
8	Linolenic acid $C_{18:3}$	$C_{18}H_{30}O_2$	1.8	1.5

As can be seen from the table, the figure for linoleic acid is much higher than that of other fatty acids. That's why *S. hypericifolia* L. could be utilized to obtain linoleic acid. As regards the qualities of linoleic acid, it is an essential fatty acid in nutrition and is used in the biosynthesis of prostaglandins and cell membranes [20].

Conclusion

In conclusion, quantitative analysis of total bioactive constituents and the moisture, total ash, organic acids, flavonoids, coumarins, saponins and tannins of *S. hypericifolia* L. were determined. Besides, macro-micro elements in the ash of the medicinal plant were investigated, and total eleven macro-micro elements were identified by the method of multi-element atomic emission spectral analysis. Meanwhile, twenty amino and eight fatty acids were determined from *S. hypericifolia* L. Of the identified amino acids of the aerial part, glutamate, aspartate and alanine predominate, while in the underground their content is slightly less. Fatty acid is mainly linoleic acid. The plants *S. hypericifolia* L. has high research potential and demands multidimensional study.

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