INVESTIGATION OF POLYPHENOLIC COMPOUNDS OF *Chamaenerion latifolium* (L.) PLANT

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ABSTRACT

In this paper, we studied the phenolic content of *Chamaenerion latifolium*. *Chamaenerion latifolium* is a plant of *Onagraceae* L. family growing in the territory of the Republic of Kazakhstan. The extraction and identification of the phenolic compounds carried out in the water, ether and acetate phase. Qualitative and quantitative analysis of flavonoids and tannins was analyzed. Using the spectrophotometry method, the amount of quercetin flavonoids (3.32% flower, 1.48% stems, 2.48% leaf,) and tannins content (5.54% flower, stem 2.76%, leaf 4.77%) were found in the plant by standard method. Results of the study showed that the higher content of flavonoids can be found in the flowers and leaves of the *Chamaenerion latifolium*. The results of the study may be helpful in the isolation of flavonoids for many purposes.

Keywords: *Chamaenerion latifolium*, Polyphenolic Compounds, Flavonoids, Tannins, Paper Chromatography.

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INTRODUCTION

Nowadays interest in plant phenolic compounds has been increasing globally. The main reason behind the significance of phenolic compounds ties with their substantial health benefits. Phenolic or polyphenolic compounds have been widely used in many areas like antioxidant and allelopathic activities,^{1,2} antimicrobial activities,³ active ingredients for cosmetic products,⁴ cosmetics,⁵ antioxidant and antitubercular drugs,^{6,7} food additives⁸ some polyphenolic compounds used for oil additives,⁹ etc.

The antioxidant properties of phenolic compounds depend on plant origin, type of solvent used in extraction, harvesting time and storage.^{10,11} The antimicrobial activity of plant extracted phenolic compounds usually have higher antimicrobial capacity.¹² There are two Holotypes of *Chamaenerion* *Adans* (*Onagraceae* Lindl. Family) in Kazakhstan: *Ch. Angustifolium* (L.) and Scop, *Ch. Latifolium* (L.).

The most popular type is *Chamaenerion angustifolium* was made into a tea known as Ivan Chai and used as a highly prized medical herb in Russia.

Chamaenerion latifolium grows all the mountains of Kazakhstan higher than 3100 meter sea level.¹³ However, phenolic compounds of *Chamaenerion latifolium* were studied very little. The study of the determination of natural phenolic compounds is very important to enhance public health. *Chamaenerion latifolium* is a good source of polyphenols, as far as our literature survey could ascertain, the composition of phenolic compounds of *Chamaenerion latifolium* has never been published. Therefore, the purpose of the research is to study of polyphenol compounds of *Chamaenerion latifolium* growing on the territory of the Republic of Kazakhstan.

EXPERIMENTAL

Extraction and Identification of *Chamaenerion latifolium* Phenol Compounds

5 g (2 mm shredded) raw material was placed in a 50 ml round-bottom flask and added to 30 ml of ethyl alcohol. Flasks were equipped with a cooler and placed in a water bath at 70°C and shaken for 15 min. After cooling, the cooled extract was filtered and the filtrate was used for qualitative analysis.¹⁴

Qualitative Determination of Flavonoids**Reaction with Aluminum Chloride**

A yellow-green color was obtained when adding 3 ml of indicator (2% AlCl_3 in an ethyl alcohol solution) into 5 ml of filtrate.

Reaction with Ammonia Vapor

A few drops of the extract were added to the filter paper, and then placed in ammonia vapor, and the flavonoids on the filter paper turned yellow. In UV light, the colors of flavonoids changed into light yellow or light green.

Quantitative Analysis of Flavonoids

Extract Preparation: 0.5 g of raw material (up to 2 mm shredded) was placed in a 100 mL round-bottom flask and 30 ml of 70% ethyl alcohol was poured into it, then the flask was boiled in a water bath for 1 hour. After cooling, the extract was filtered through a paper filter and filtrates were placed into a 100 ml measuring flask. The extraction was repeated. Then the flask was filled with 70% ethyl alcohol.¹⁵

Preparation of a Standard Solution

0.0005g of quercetin dissolved in 50 ml of water. In 50 ml measuring flasks 0.1; 0.2; 0.4; 0.8; 1.0; 1.2 ml of standard solutions were prepared.

Analysis of Samples

1.5 ml of 96% alcohol, aluminum chloride, 1 ml of potassium acetate and 2.8 ml of water were added in water-alcohol extract (0.5 mg/ml). After 30 minutes infusions from *Chamaenerion latifolium* were analyzed by spectrophotometer. Measurements were performed on an Agilent Cary 60 instrument with a wavelength of 410 nm and a thickness of 10 mm of the cuvette.

Preparation of a Standard Solution

25 ml measuring flask filled with 0.005 g of a standard substance, quercetin and 50% ethyl alcohol. The formula has been used to calculate the quantity (X,%) of flavonoids in the raw materials:

$$X = C * 100 * 10 * 100 * 6 / m * 1 * 1000 * (100 - W)$$

C – the amount of flavonoids, found from the calibration graph, mg

m – mass of the sample, g

W – lost mass of samples during drying, %

Methods of Extraction of Plant Raw Materials

1 g of 2 mm crushed raw material was poured into 100 ml of boiled distilled water, then placed in a 150 ml flask and heated in a water bath for 25-30 min. After cooling, the extract was filtered and the filtrate was used for qualitative reactions.

Reaction with TAA Solution

Add 2-3 drops of iron-ammonium starch (TAA) solution to 2-3 ml of extract, the hydrolyzed agents turn dark blue, and the condensed substances are colored dark green or precipitate. This reaction is often used for qualitative analysis in many agents extracted from plants.

The 1% solution of vanillin in concentrated sulfuric acid gives red color when reacted with condensed tannin. No color change was detected, when 1 ml of 1% *Chamaenerion latifolium* extracts was added to vanillin solution in concentrated sulfuric acid.

Extract Preparation

2.0 g of the raw materials (finely ground up to 2 mm) was placed into a 500 ml round-bottom flask, added 250 ml of distilled water and heated for 30 min in a water bath. The hot extract is filtered and returned into 250 ml measuring flask and filled with distilled water to the flask mark.^{15,18}

Preparation of Standard Solution

50 mg of tannin was dissolved in 50 ml of water. 0.1; 0.2; 0.4; 0.8; 1; 1.2 ml of standard solution was prepared.

Performing the Analysis

1 ml of aliquot extract was poured into a 50 ml measuring flask, then 1 ml of 2% aqueous solution of ammonium molybdenum acid was added, the flask filled with distilled water. After 15 minutes the optical density of the solution was measured on an Agilent Cary 60 device with a 10 mm thick cuvette at a wavelength of 420 nm.

Preparation of Standard Solution¹⁶

The aliquot of the 1 ml extract was poured into a 50 ml measuring flask and distilled water. The percentage of solids was calculated by the following formula (X,%):

$$X = C \cdot 100 \cdot 100 / m \cdot 10 \cdot (100 - W)$$

C – the amount of flavonoids as quercetin, found from the calibration graph, mg;

m – a mass of the sample, g;

W – lost mass of samples during drying, %;

RESULTS AND DISCUSSION

Isolation and Identification of Phenolic Compounds of *hamaenerion latifolium*.

The extracted polyphenols are investigated in Butanol-Acetic acid-Water (BAW) system by special indicators (AlCl₃, Ferric ammonium sulfate, FeCl₃ in ammonium vapor) using 2-D paper chromatography.¹⁷ The chromatogram of the *Chamaenerion latifolium* in water medium (flower, leaves, stems) is shown in Fig.-1.

Fig.-1: The Chromatogram of *Chamaenerion latifolium* (a) Flower; (b) Leaves; (c) Stems.

The R_f values and colors for the *chamaenerion latifolium* flower, leaf, and stem sections are given in Table-1.

Table-1: Specific Reactions of *Chamnaerion latifolium* Extract (Flower, Leaf and Stem)

No.	Flower					
	R _f		Quality Reaction			
	BAW	HAc	NH ₃	AlCl ₃	Ferric ammonium sulfate	FeCl ₃
1	0,82	0,70	Light yellow	Dark yellow	Gray	Gray
2	0,81	0,67	Yellow	Yellow	Blue	Blue
3	0,79	0,55	Yellow	Dark yellow	Green	Blue
4	0,80	0,65	Yellow	Yellow	Blue	Deep blue
5	0,20	0,70		-	Blue	Deep blue
6	0,10	0,67	-	-	Blue	Deep blue

7	0,9	0,55	-	-	Blue	Dark gray
8	0,18	0,65	-	-	Blue	Deep blue
9	0,21	0,45	-	-	Gray	Deep blue
10	0,15	0,25	-	-	Blue	Deep blue

Leaf						
No.	R _f		Quality Reaction			
	BCC	HAc 15%	NH ₃	AlCl ₃	Ferric ammonium sulfate	FeCl ₃
1	0,72	0,61	Light yellow	Dark yellow	Gray	Gray
2	0,71	0,57	Yellow	Yellow	Dark green	Dark green
3	0,70	0,50	Yellow	Dark yellow	Dark green	Dark green
4	0,69	0,63	Yellow	Yellow	Blue	Deep blue
5	0,20	0,70		-	Blue	Deep blue
6	0,10	0,67	-	-	Blue	Deep blue
7	0,9	0,55	-	-	Blue	Deep blue
8	0,18	0,65	-	-	Blue	Deep blue

Plant Stem						
No.	R _f		Quality Reaction			
	BCC	HAc 15%	NH ₃	AlCl ₃	Ferric ammonium sulfate	FeCl ₃
1	0,41	0,33	Yellow	Dark yellow	Gray	Gray
2	0,35	0,43	Yellow	Yellow	Dark green	Dark green
3	0,65	0,51	Yellow	Dark yellow	Dark green	Gray - blue
4	0,58	0,33	Yellow	Yellow	Blue	Deep blue
5	0,21	0,55		-	Blue	Deep blue
6	0,10	0,66	-	-	Blue	Deep blue
7	0,11	0,54	-	-	Blue	Dark gray

According to the chromatogram, the flowers of the plant contains a large amount of tannin substances. The chromatogram of the leaf of *Chamaenerion latifolium* in water medium is shown in Fig.-1 and Table-1. Various reagents were used to separate the total plant extract: ether, ethyl acetate, and water. In ether phase immobile aglycons, phenolic carbon acids. The chromatogram of *Chamaenerion latifolium* in ether phase is shown in Fig.-2.

Fig.-2: The Chromatogram of *Chamaenerion latifolium* in ether Phase (a) Flowers, (b) Leaves and (c) Stems

According to the Chromatographic results, the yellow-colored substances were flavonoids and blue-colored phenolcarbon acids.^{19,20} Chromatography is also used to separate flavonoids into individual components, the results of which are shown in Table-2.

Table-2: The Chromatogram of *Chamaenerion latifolium* in Ether Phase

№	R _f		Quality Reaction			
	BAW	HAc 15%	NH ₃	AlCl ₃	Ferric ammonium sulfate	FeCl ₃
Flower						
1	0,82	0,21	Yellow	Dark yellow	Gray	Gray
2	0,81	0,41	Yellow	Yellow	Green	Green
3	0,78	0,39	Yellow	Dark yellow	Green	Green
4	0,77	0,10	Yellow	Yellow	Green	Green
5	0,77	-		-	Blue	Deep blue
6	0,66	0,10	-	-	Blue	Deep blue
7	0,55	-	-	-	Blue	Deep blue
8	0,49	-	-	-	Blue	Deep blue
Leaf						
1	0,81	0,31	Yellow	Dark yellow	Gray	Gray
2	0,71	0,21	Yellow	Yellow	Green	Green
3	0,66	0,27	Yellow	Dark yellow	Green	Green
4	0,55	0,17	Yellow	Yellow	Green	Green
5	0,43	0,10		-	Green	Green
6	0,33	-	-	-	Blue	Deep blue
7	0,31	-	-	-	Blue	Deep blue
Plant Stem						
1	0,83	0,31	Yellow	Dark yellow	Gray	Gray
2	0,69	0,21	Yellow	Yellow	Green	Green
3	0,66	0,27	Yellow	Dark yellow	Green	Green
4	0,55	0,17	Yellow	Yellow	Green	Green

The ether part was analyzed by a two-dimensional chromatography system. The two-dimensional paper chromatography of the ether extract showed the presence of quercetin, gallic acid. The ether extracts were identified by AlCl₃, Ferric ammonium sulfate, FeCl₃ indicators. As a result, it was found that the yellow substance quercetin is present in the flower, stem, leaf, and gallic acid only in the leaf and flower. Correspondence of State standard with quercetin was observed. Chromatographic data can be found in Fig.-3 and Table-3.

The results of the chromatography with the quercetin by state standard in Butanol-Acetic acid-Water system are presented in Table-3.

Fig.-3: (a) Reaction with Quercetin and Labeling; (b) Reaction with the Gallic Acid Following State Standard

Table-3: Chromatogram Results with Quercetin by State Standard and Chromatogram of the Gallic Acid

No.	Parts	R _f	Color	No.	Color	R _f	Ferric ammonium sulfate	FeCl ₃
1.	Leaves	0.83	Yellow	1.	Leaves	0.55	Bluish gray	Blue
2.	Stems	0.81	Light Yellow	2.	Stems	-	-	-

3.	Quercetin, state standard	0.88; 0.75	Dark Yellow	3.	Gallic acid following state standard	0.50	Dark blue	Dark Blue
4.	Flower	0.93; 0.79; 0.68	Yellow, Yellow, Yellow	4.	Flowers	0.52 0.53	Bluish gray	Light Blue

In the HAc 15% system, Ferric ammonium sulfate and 1% FeCl₃ solution were used. Correspondence of State standard with gallic acid was observed. The chromatographic data are shown in Fig.3 and Table 3.

According to the chromatogram of the etheric extract with gallic acid, it was found that there was no phenolic carbonic acid in the stem.

Study of the ethyl acetate phase. Ethyl acetate phase was separated and placed in the 2-D chromatography system. Based on the ethyl acetate chromatogram, we can assume that the flavonoids and phenol carboxylic acids have been released into the ether. By the paper chromatography method, ethylacetate was placed in the 2-D (BAW; 15% HAc) system and identified by special indicators (ammonia vapor, AlCl₃; Ferric ammonium sulfate; FeCl₃). Identification was performed on the aboveground parts of the plant (flowers, leaves, stems). The chromatogram of *Chamaenerion latifolium* ethyl acetate fragment is shown in Fig.-4.

Fig.-4: Chromatogram of Ethylacetate phase of *Chamaenerion latifolium* (Flowers, Leaves, Stems)

Blue spots-tannin by chromatographic result. The results are presented in Table-4.

The chromatogram of ethyl acetate phase of *Chamaenerion latifolium*, corresponding to tannin and purity of tannin was checked by paper chromatography. According to the results, the tannin content was in the order of Flowers > Leaves > Stems, which means the flower has more tannin than leaf and stem. Ferric ammonium sulfate and 1% FeCl₃ solution were used for identifying in the HAc 15% system. Values and colors are shown in Table-5.

Table-4: Indicating Reaction of Ethylacetate Phase

№	R _f		Quality reaction			
	BAW	HAc	NH ₃	AlCl ₃	Ferric ammonium sulfate	FeCl ₃
Flower (a)						
1	0.32	0.66	Light Yellow	Saturated Yellow	Gray	Blue
2	0.41	0.65	Yellow	Yellow	Blue	Blue
3	0.45	0.55	Yellow	Saturated yellow	Gray blue	Gray
4	0.46	0.49	Yellow	Saturated yellow	Gray Blue	Gray
5	0.46	0.77	Yellow	Yellow	Blue	Blue
Leaf (b)						
1	0.32	0.64	Light yellow	Saturated Yellow	Gray	Gray

2	0.40	0.63	Yellow	Yellow	Blue	Blue
3	0.40	0.53	Yellow	Saturated Yellow	Gray Blue	Blue
4	0.46	0.76	Yellow	Yellow	Blue	Blue
Plant Stem (c)						
1	0.32	0.66	Light Yellow	Saturated Yellow	Gray	Gray
2	0.41	0.65	Yellow	Yellow	Blue	Blue
3	0.45	0.55	Yellow	Saturated yellow	Gray blue	Gray blue

Table-5: Chromatogram of Tannin by State Standard

Indicators	Rf
Ferric ammonium sulfate Indicator	0.55
FeCl ₃ Indicator	0.54

Ferric ammonium sulfate and 1% FeCl₃ solution were used for identifying tannin in the HAc 15% system. The chromatographic data were shown in Fig.-5 and Table-6. Identification was performed on the aboveground parts of the plant (flowers, leaves, stems).

Fig.-5: The Chromatogram of Ethyl acetate Phase of *Chamaenerion latifolium*Table-6: The Chromatogram of Ethyl acetate Phase of *Chamaenerion latifolium*

	Rf	Ferric ammonium sulfate	FeCl ₃
Leaves	0.65 0.71	Grey blue	Light blue
Stems	0.70	Dark blue	Dark blue
Tannin (state standard)	0.70	Dark blue	Dark blue
Flowers	0.65 0.70 0.75	Grey blue Dark blue Dark blue	Blue Blue Blue

Defining the structure of flavonoids and their glycosides begin with qualitative reactions. These are the reduction reaction (magnesium or zinc dust in hydrochloric acid, sodium borohydride), the complex reaction, and the change in the color of the reaction with alkali or iron chloride.

Most often qualitative reactions are used in combination with chromatography (paper chromatography). This method is widespread because the solubility of flavonoids is different and they are painted in unusual colors when detecting flavonoids in ultraviolet or visual light with complex reagents.¹⁷

Complex Reaction

In a specific reaction with aluminum chloride, flavonoids form yellow complexes. The spectrophotometric method was used to determine the quantitative content of flavonoids, and quercetin was taken as the standard. Flavonoids give a yellow reaction to aluminum chloride or yellow-green fluorescence. The caliber of the Quercetin standard is shown in Figure 6, and the quantity of flavonoids in Table-7.

It has been proven that *Chamaenerion latifolium* has a large amount of flavonoids in flowers. Qualitative determination of tannin in the raw material was performed by the standard method. All tannin react with *Ferric ammonium sulfate*. The hydrolyzed tannin gives a dark blue reaction and the condensed tannin give a dark green reaction. This reaction is used to extract tannins from plant raw materials.

When 1% solution of vanillin in concentrated sulfuric acid gives no color when added into 1 ml of *Chamaenerion latifolium* extract, which means there were no condensed tannins.

Chamaenerion latifolium hydrolyzed tannins were determined during qualitative reactions in plant raw materials.

Fig.-6: (a) Caliber with Quercetin Standard; (b) Calibration Curve of Tannin Standard

Table-7: Quantitative Quantities of Flavonoids and Analysis of Tannin in the *Chamaenerion latifolium*

Parts of the Plant	Mass of the Sample, g	Optical Density (Flavonoids)	Quantities of Flavonoids, %	Optical Density (Tannin)	Quantities of Tannins, %
Flowers	1.000	0.4992	3.32	0.4999	5.54
Leaves	1.000	0.3861	2.48	0.4448	4.77
Stems	1.000	0.2211	1.48	0.2471	2.76

Quantitative analysis of tannin in aqueous extract of *chamaenerion latifolium* was performed by spectrophotometric method. The reaction of the tannins with the ammonium molybdate is carried out by the following reaction:

The resulting product $\text{C}_6\text{H}_3(\text{OH})_2\text{OMoO}_2(\text{NH}_4)_2$ turns the reaction medium to yellow, which allows the quantitative measurement of tannin substances by spectrophotometric method. The tannin standard caliber graph was shown in Fig.-6. The tannin in the *Chamaenerion latifolium* was given in Table-7.

It was found that the largest amount of flavonoids and tannins in *Chamaenerion latifolium* is found in the flower of the plant.

CONCLUSION

Phenolic compounds of the Holotypes of *Chamaenerion Adans* (*Onagraceae Lindl. Family*) *Chamaenerion latifolium* L. were identified. Qualitative and quantitative analysis of flavonoids and tannins was analyzed. Using the spectrophotometry method, the amount of quercetin flavonoids was found in the plant by the standard method (3.32% flower, 1.48% stems, 2.48% leaf), tannins content (5.54% flower, stem 2.76%, leaf 4.77%).

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