

## Chemical composition of *Gaillardia pulchella* foug (asteraceae) inflorescences

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### Abstract

This article presents information on antioxidant activity, flavonoids, polysaccharides, amino acids and tannins of *Gaillardia pulchella* inflorescences. The aim of this work is to evaluate the chemical composition of the *Gaillardia pulchella* Foug inflorescences. The quantitative content of flavonoids and antacians was carried out by spectrophotometrical method. The content of pectin substances was determined by the M. Sinner method. Quantitative analysis of tannins was carried out permanganometrical method. The maximum content of the antioxidants was revealed in the 70% ethanolic extract of *Gaillardia pulchella* inflorescences. The content of water-soluble polysaccharides and pectin in the inflorescence *Gaillardia pulchella* meal is 4.5% and 5.1%, respectively. The sum of flavonoids in *Gaillardia pulchella* extract is  $0.32 \pm 0.01\%$ . Based on the conducted qualitative reactions, condensed tannins are contained in the aqueous extract. In the water extract of *Gaillardia pulchella* inflorescences, aspartic and glutamic acids,  $\alpha$ -alanine,  $\alpha$ -proline, methionine and valine were found. Based on the obtained data, potassium predominates in the 70% ethanolic extract of *Gaillardia pulchella* inflorescences. In the result of this work, flavonoids, polysaccharides, amino acids, micro-, macroelements composition, and tannins of inflorescences *Gaillardia pulchella* were evaluated.

**Keywords:** inflorescences *Gaillardia pulchella*, flavonoids, tannins, amino acids and polysaccharides

### 1. Introduction

The search for biologically active compounds among new raw plant materials previously unused in medical practice is an urgent problem. *Gaillardia pulchella* Foug. Belongs to the family Asteraceae. The sesquiterpenes of the lipophilic fraction of inflorescences *Gaillardia pulchella* were studied <sup>[1]</sup>. Essential oil from *Gaillardia pulchella* Foug. Flowers was obtained by hydrodistillation, and its chemical composition was analyzed by chromatography-mass spectrometry (GC-MS). 28 compounds were identified, of which the most famous were n-hexadecanoic acid, phytol and cyclopropanooctanoic acid, 2 - [[2 - [(2-ethylcyclopropyl) methyl] cyclopropyl] methyl] -, methyl ether <sup>[2]</sup>. We experimentally established antihypoxic and anti-ischemic properties of *Gaillardia pulchella* inflorescences extract obtained by extraction of 70% ethyl alcohol. Thus, in conditions of experimental focal cerebral ischemia, the therapeutic administration of extract from *Gaillardia pulchella* helps to reduce the size of the brain necrosis zone by stabilizing mitochondrial function and reducing glycolytic processes. In addition, the restoration of cellular respiration processes probably contributes to an increase in the life span of animals under various pathogenetic types of hypoxia were noted <sup>[3]</sup>. Extract from inflorescences of *Gaillardia pulchella*, obtained by extraction with ethyl alcohol of 70%, *in vitro* tests also suppresses the generation of superoxide, nitrosyl, hydroxyl, and DPPH radicals, which may indicate a high antioxidant potential of this extract <sup>[4]</sup>. The object of the study was the beautiful *Gaillardia* inflorescences, collected in July - August 2017 - 2018, in the Caucasus Mineral Water region. All of the above was the basis for further study of the chemical composition of biologically active compounds of *Gaillardia*

*pulchella* Foug inflorescences, which is the aim of this work.

### 2. Materials and methods

#### 2.1. Determination of the total content of antioxidants

The total content of antioxidants was determined on a liquid chromatograph "Tzvet Yauza-01-AA". The mass concentration of antioxidants is measured using a calibration graph of the dependence of the output signal on the concentration of quercetin and / or gallic acid <sup>[5, 6, 7, 8]</sup>.

#### 2.2 The study of flavonoids

##### 2.2.1 Preliminary chromatographic analysis

The alcohol and water-alcohol extracts obtained by extraction of the raw materials were analyzed by paper chromatography in the solvent system n-butanol - acetic acid - water (4: 1: 5) and 15% acetic acid. Detection of components in the chromatogram was carried out by viewing them in UV light. After viewing in UV light and subsequent treatment with the following reagents: 5% alcohol solution of AlCl<sub>3</sub> (bright yellow color of the adsorption zone in visible light and bright yellow-green fluorescence in UV light) and 5% aqueous solution of FeCl<sub>3</sub> (green - yellow color of the adsorption zone in visible light and bright green fluorescence in UV light) <sup>[9, 10, 11]</sup>.

##### 2.2.2 Analysis of the *Gaillardia pulchella* Foug inflorescences 70% ethyl alcohol extract by fraction

Extracts from *Gaillardia pulchella* inflorescences were obtained by extraction of the raw material with ethyl alcohol 70% in a water bath in a flask with a reflux condenser (extraction ratio 3, duration 1 hour). This extract was evaporated until the ethyl alcohol was completely removed, after which the residue was successively treated with

chloroform, diethyl ether, ethyl acetate, butanol, followed by chromatographic analysis of these four fractions.

### 2.2.3 Quantitative determination of flavonoids

The quantitative content of flavonoids was determined spectrophotometrical method. The absorbance of the solution was measured on an «Aquilon» spectrophotometer at a wavelength of 400 nm. The percentage of flavonoids in terms of quercetin and absolutely dry raw materials is calculated by the formula [9]:

$$X_1 = \frac{A \cdot 25 \cdot 100 \cdot 100}{A_{1\text{cm}}^{1\%} \cdot a \cdot 2 \cdot (100 - W)} ; \quad (1)$$

where: A – is the optical density of the test solution;  $A_{1\text{cm}}^{1\%}$  – is the specific absorption coefficient of the complex of luteolin with aluminum chloride at a wavelength of 400 nm, 549; a – a sample of raw materials, g; W – is the moisture content of the raw material, %.

### 2.2.4. Determination of antacians

The determination of antacians was carried out in terms of cyanidin-3-O-glucoside. The absorbance of the solution was measured on «Aquilon» spectrophotometer at a wavelength of 534 nm using the specific absorption coefficient of the complex of cyanidin-3-O-glucoside with aluminum chloride [9]:

$$X_1 = \frac{A \cdot 25 \cdot 100 \cdot 100}{A_{1\text{cm}}^{1\%} \cdot a \cdot 2 \cdot (100 - W)} ; \quad (2)$$

where: A – is the optical density of the test solution;  $A_{1\text{cm}}^{1\%}$  – A – оптическая плотность исследуемого раствора;  $A_{1\text{cm}}^{1\%}$  – specific absorption coefficient of the complex of cyanidin-3-O-glucoside with aluminum chloride at a wavelength of 534 nm, 100; a – sample of the raw material, g; W – moisture content of the raw material, %.

## 2.3 The study of the polysaccharide complex

### 2.3.1 Isolation of polysaccharides and the study of monosaccharide composition

Polysaccharides were determined gravimetrically by N.K. Kochetkov and M. Sinner [12, 13, 14, 15]. The monosaccharide composition of the obtained fractions after hydrolysis with a 10% solution of sulfuric acid at 100 ° C for water-soluble polysaccharides for 10 hours and for the remaining polysaccharide complexes within 48 hours was identified by chromatographic method. For this purpose, the hydrolysates were neutralized barium carbonate powder using a universal indicator to a neutral medium, filtered, evaporated in a water bath to a small residue, and chromatographed [14, 15, 16, 17]. The solvent phase was the n-butanol – acetic acid – water (4: 1: 5) solvent system (developer aniline phthalate reagent), and the stationary phase was Munktell Chrom.-Paper Sheets Grade paper. FN7 (Germany) [12, 13, 14].

### 2.3.2 Definition of functional groups

The quantitative determination of the functional groups of pectin substances (free carboxyl, methoxylated carboxyl, the total number of carboxyl, as well as the content of methoxy groups) was carried out by the alkalimetric method (sodium

hydroxide solution (0.1 mol / l) [18, 19, 20, 21].

## 2.3.4 Determination of amino acid composition

### 2.3.4.1 Preliminary chromatographic analysis

The identification of amino acids in the *Gaillardia pulchella* Foug inflorescences extract obtained by extraction with purified water was carried out by paper chromatography in the presence of standard samples. Chromatographic paper grade FN-7 (Germany) was used as the stationary phase. The solvent systems used were the mobile phase: n-butanol – acetic acid – water (4: 1: 5) and acetone – water (3: 2). A ninhydrin alcohol solution was used as a developer [11, 22].

## 2.4.5 Electrophoretic determination of amino acid and elemental composition

The content of free and bound amino acids, as well as the elemental composition was determined by capillary electrophoresis on an electrophoretic device «Kapel» No. 17727-01. The technique is based on the separation of the anionic forms of N-phenylthiocarbonyl derivatives of amino acids under the influence of an electric field due to their different electrophoretic mobility. To identify and quantify the analyzed components, ultraviolet absorption is recorded at a wavelength of 254 nm [23, 24, 25].

## 2.5 The study of tannins

### 2.6.1 Qualitative analysis

To conduct a qualitative analysis of tannins from the analyzed raw materials, aqueous extracts were prepared (1:10). Tannins form colored complexes with salts of heavy metals and precipitates with high molecular weight compounds, and also combine reactions with diazonium compounds [9, 26, 27].

### 2.6.2. Quantitative analysis

The permanganometric method was used to quantify tannins in the obtained extract. This method has a number of disadvantages, since other compounds with a similar chemical structure are oxidized, and therefore, we also used the deposition of tannins with gelatin [9].

## 3. Results and discussion

### 3.1 Determination of the total content of antioxidants

The moisture content of *Gaillardia pulchella* inflorescences extract is 8.29% [9].

In alcohol and water-alcohol extracts, antioxidants were determined by the peak area of the differential curves of the corresponding extracts. The peak areas, as well as the concentration of antioxidants in terms of quercetin and gallic acid are presented in table 1.

## 3.2 The study of flavonoids

### 3.2.1 Chromatographic analysis

The alcohol and alcohol-water extracts obtained from the raw materials were used for paper chromatography in the solvent system n-butanol - acetic acid - water (4: 1: 5) and 15% acetic acid. Detection of components in the chromatogram was carried out by viewing them in UV light. It was found that the clearest separation is observed when using 15% acetic acid, which indicates the glycosidic nature of the compound. The greatest amount of phenolic compounds is observed in the extraction obtained with ethyl alcohol of 70% (from 4 to 5 adsorption zones).

### 3.2.2 Separation of the extraction from *Gaillardia pulchella* obtained with ethyl alcohol 70% into fractions

Subsequently, the obtained extract was subsequently treated with chloroform, diethyl ether, ethyl acetate, butanol. The data of chromatographic analysis of four fractions in two systems are presented in table 2.

From the presented data it follows that the obtained extracts are characterized by a minimum content of polyphenolic compounds.

### 3.2.3 Quantification of flavonoids

The absorption maximum of the analyzed extract with a 2% alcohol solution of aluminum chloride corresponds to a value of  $400 \pm 2$  nm, which coincides with that of luteolin. The complex of luteolin and the extraction of inflorescences *Gaillardia pulchella* obtained by extraction with 70% ethyl alcohol, with a 2% alcohol solution of aluminum chloride, have a maximum absorption at the same wavelength of  $400 \pm 2$  nm (Figure 1). Therefore, when calculating the total flavonoid content, the specific absorption coefficient of the complex compound of luteolin with aluminum chloride was used [9].

The results obtained are presented in table 3. The content of the sum of flavonoids in the inflorescences *Gaillardia pulchella* was  $0.32 \pm 0.0101\%$ .

Due to the fact that the reaction develops in time, we found that the optical density is stabilized for 45 minutes, which is optimal for analysis (table 3).

UV absorption spectrum of a complex of 70% alcohol extracts from inflorescences *Gaillardia pulchella* with a 2% alcohol solution of aluminum chloride in timeline represented in figure 2.

### 3.2.4 Determination of antacians

The study of antacians was carried out spectrophotometrically in terms of cyanidin-3-O-glucoside at a wavelength of 534 nm [9].

The sum of antacians in the inflorescences of *Gaillardia pulchella* was  $3.41 \pm 0.0355\%$ .

## 3.3 The study of the polysaccharide complex

### 3.3.1 Isolation of polysaccharides and the study of their monosaccharide composition

The content of the polysaccharide complex was determined gravimetrically. The results are presented in table 5.

In the processing of plant materials in order to obtain polysaccharide complexes, extraction is used with purified water, as well as with aqueous solutions of ammonium oxalate, oxalic acid and sodium hydroxide. As a rule, in order to preserve other important biologically active compounds, in particular polyphenols, the raw materials are pre-treated with organic solvents, and then polysaccharides are obtained from the remaining meal [14, 15, 16].

Gravimetric analysis indicates the predominance of pectin substances in the inflorescences of *Gaillardia pulchella* 5.12%. The PV fraction isolated from the inflorescences *Gaillardia pulchella* contains glucose, galacturonic acid, xylose and rhamnose.

### 3.2 The quantitative content of free functional groups.

The study of the qualitative characteristics of pectin substances and the determination of functional groups is of

interest to justify the possibility of their use for medical purposes [20, 21].

From the table it follows that the content of free carboxyl groups of pectin substances is 20.61%, and the degree of etherification is 6.15%.

All studied pectin substances belong to the group of low esterified pectin's, because characterized by a low degree of etherification. A significant content of free carboxylate groups indicates their rather high complex-forming ability.

## 3.4 Determination of amino acid composition

### 3.4.1 Preliminary chromatographic analysis

Amino acids were identified by extracting from *Gaillardia pulchella* inflorescences obtained by extraction with purified water by paper chromatography when compared with reliable witness samples. After processing by ninhydrin reagent, the presence of blue-violet spots was observed [11, 22].

The intensity of adsorption zones in the chromatograms revealed: glutamic acid, glycine and valine.

### 3.4.2 Electrophoretic determination of amino acids

The content of free and bound amino acids was determined by capillary electrophoresis on an electrophoretic «Kapel» device No. 17727-01 [23, 24, 25].

The spectrograms show the content of free amino acids in the extract from the inflorescences *Gaillardia pulchella* obtained by extraction with purified water.

Thus, 12 free amino acids were found in the analyzed extract, of which 6 are essential (arginine,  $\beta$ -phenylalanine, leucine, methionine, threonine, valine). The largest amounts contain methionine, valine, proline, glutamic and aspartic acids.

## 3.5 Determination of elemental composition

The elemental composition in extracting inflorescences *Gaillardia pulchella* obtained by extraction with ethyl alcohol of 70% was determined by capillary electrophoresis on an electrophoretic «Kapel» device No. 17727-01 [23, 24, 25]. Information about the elemental composition of the analyzed extract is presented in Figure 5 and in table 9.

Based on the data obtained, potassium prevails in the extraction of inflorescences *Gaillardia pulchella* obtained by extraction with ethyl alcohol 70%.

## 3.6 The study of tannins

### 3.6.1 Qualitative analysis

To conduct a qualitative analysis of tannins from air-dried raw materials, aqueous extracts were prepared (table 10).

### 3.6.2. Tannins quantitative determination.

Quantitative determination of tannins was carried out permanganometrically and the reaction of deposition of tannins with gelatin was used [9].

When using different methods to determine the amount of tannins in the raw material, different results are obtained. The method of permanganometry is traditional for the quantitative determination of tannins, but it has several disadvantages in the ability of potassium permanganate to oxidize, including many natural compounds belonging to different classes.

**Table 1:** The content of antioxidants (in terms of quercetin and gallic acid) in extracts from inflorescences of *Gaillardia pulchella* Foug.

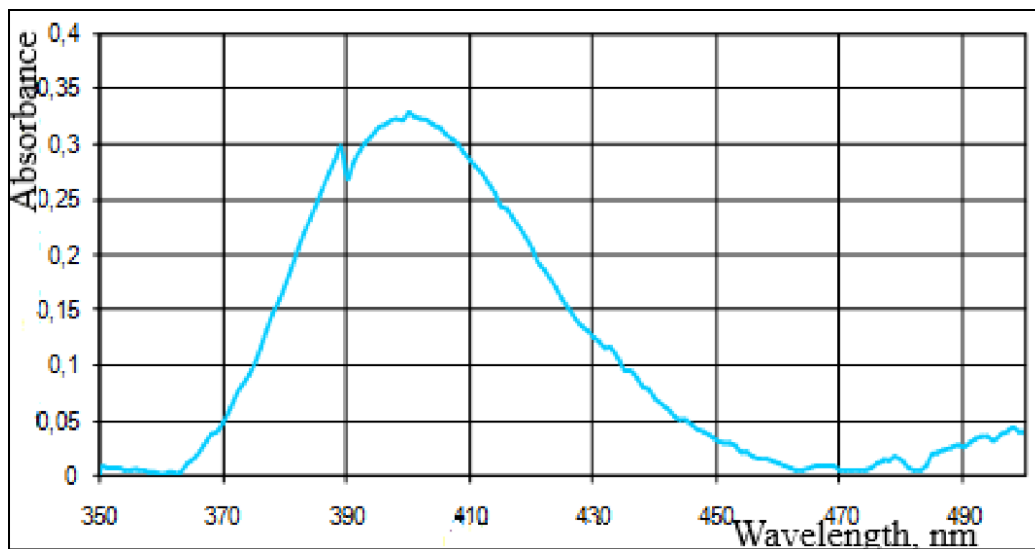
Name of raw material	Used extractants	Peak Area (Sp nA/s)	The dilution rate of the analyzed sample	The content of antioxidants (in terms of quercetin, mg/g)	The content of antioxidants (in terms of gallic acid, mg/g)
Inflorescences of <i>Gaillardia pulchella</i> Foug.	ethyl alcohol 95%	4012.77	4	1.39±0,006	0.90±0,007
	ethyl alcohol 70%	5419.88	4	1.89±0,015	1.24±0,009
	ethyl alcohol 40%	4968.36	4	1.72±0,009	1.13±0,005
	purified water	3632.98	4	1.25±0,0010	0.81±0,007

**Note:** Extracts containing the maximum amount of antioxidants are shown in bold italics.

Based on the experimental data presented in table 1, we can conclude that the maximum content of the amount of antioxidants was detected in extracts from the inflorescences of *Gaillardia pulchella* obtained by extraction with ethyl alcohol 70%.

**Table 2:** Chromatographic analysis of the obtained fractions

The investigated fractions	The value of the mobility coefficients	Before processing (visible light)	Before processing (UV light)	Before processing AlCl <sub>3</sub> (visible light)	Before processing AlCl <sub>3</sub> (UV light)
		Coloring of adsorption zones (solvent system - 15% acetic acid)			
Chloroform extraction	1) 0.22; 2) 0.48.	1) light yellow; 2) colorless.	1) dark brown; 2) blue;	1,2) yellow.	1) bright yellow; 2) bright blue.
Ethereal Extraction	1) 0.25; 2) 0.60.	1) light yellow; 2) colorless.	1) dark brown; 2) blue;	1) bright yellow; 2) colorless.	1) tan; 2) bright blue.
Ethyl Acetate Extraction	1) 0.11; 2) 0.25; 3) 0.63; 4) 0.75; 5) 0.83.	1,2,3) light yellow; 4,5) colorless.	1,2,3) yellow; 4) purple; 5) blue.	1,2,3) bright yellow; 4,5) light yellow.	1,2,3) bright yellow; 4) bright purple; 5) bright blue.
Butanol Extraction	1) 0.11; 2) 0.54; 3) 0.79.	1,2) light yellow;; 3) colorless	1) light yellow; 2) dark brown; 3) blue.	1,2) bright yellow; 3) light green.	1) light yellow; 2) light brown; 3) bright blue.
70% alcohol extraction from <i>gaillardia</i>	1) 0.05; 2) 0.21; 3) 0.50; 4) 0.73; 5) 0.81.	1-4) light yellow; 5) colorless.	1) yellow; 2) dark brown; 3,4) light yellow; 5) blue.	1-4) bright yellow; 5) colorless.	1,3,4) bright yellow; 2) tan; 5) bright blue.



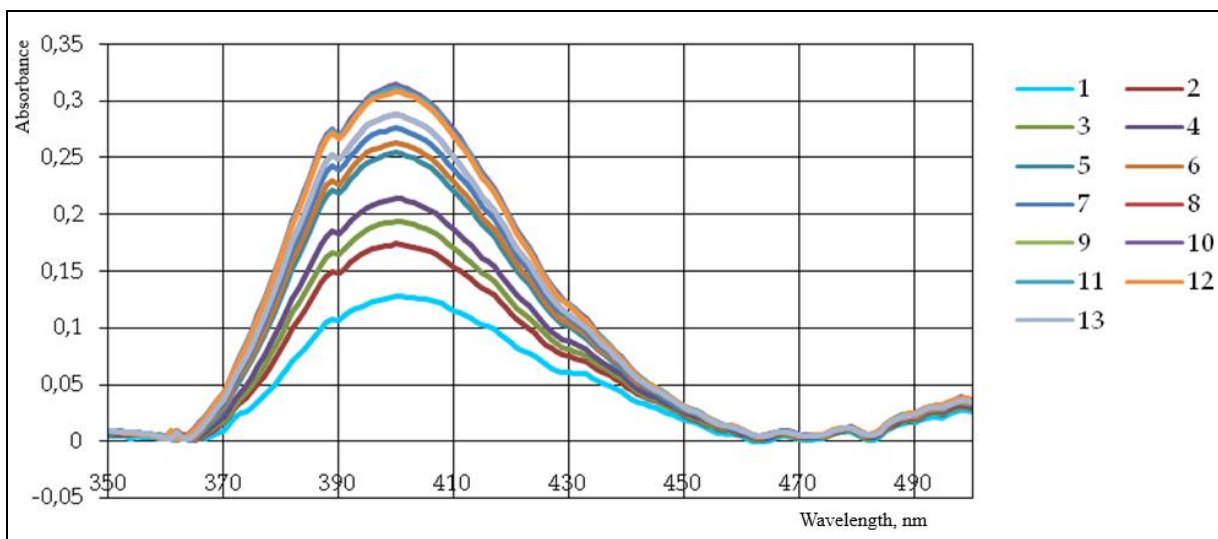
**Fig 1:** UV absorption spectrum of a complex of 70% alcohol extraction from inflorescences *Gaillardia pulchella* with 2% alcohol solution of aluminum chloride

**Table 3:** The content of the sum of flavonoids in the extracts of inflorescences *Gaillardia pulchella* obtained by extraction with ethyl alcohol 70%

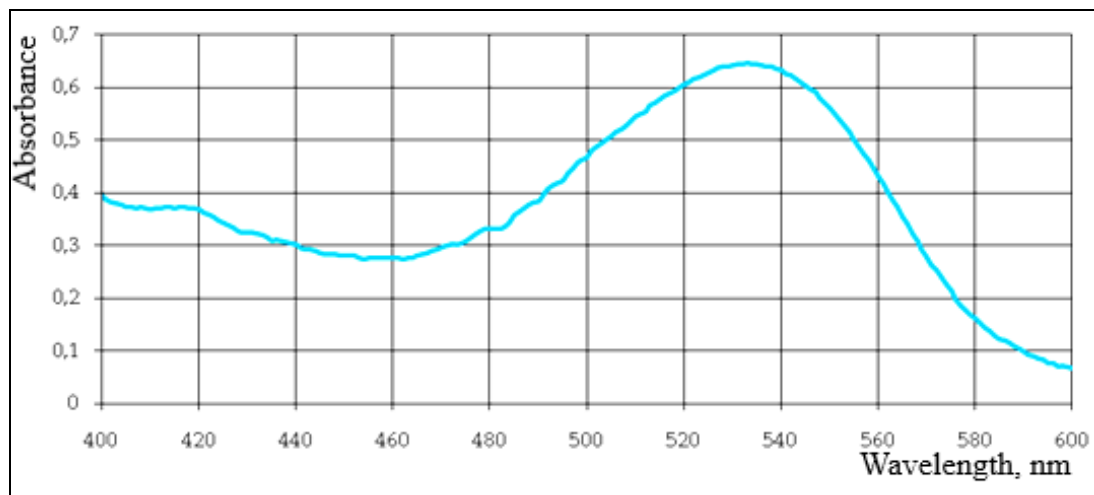
A portion of raw materials, g	Optical density	The amount of flavonoids,%	Metrological characteristics X =0.32 S =0.0039 Δx=0.0101 E= 3.12%
1.0018	0.326	0.318	
1.0094	0.349	0.339	
1.0120	0.335	0.324	
1.0025	0.329	0.321	
1.0164	0.342	0.329	
1.0007	0.318	0.311	

**Table 4:** Studying the color stability of a complex for extracting inflorescences *Gaillardia pulchella* with aluminum chloride solution

Designation	Exposure time, min	Optical density	Value Flavonoid content, %
1	5	0.129	0.126
2	10	0.175	0.171
3	15	0.196	0.191
4	20	0.216	0.211
5	25	0.257	0.251
6	30	0.265	0.259
7	35	0.278	0.272
8	40	0.323	0.316
9	45	0.326	0.318
10	50	0.317	0.310
11	55	0.314	0.307
12	60	0.311	0.304
13	65	0.291	0.284



**Fig 2:** UV absorption spectra of a complex of 70% alcohol extracts from inflorescences *Gaillardia pulchella* with a 2% alcohol solution of aluminum chloride in time (every 5 min)



**Fig 3:** UV absorption spectrum of the extraction of inflorescences *Gaillardia pulchella* obtained by extraction of 95% ethyl alcohol containing hydrochloric acid

**Table 5:** Information on the obtained polysaccharide fractions from meal of inflorescences of *Gaillardia pulchella* Foug.

Fractions	Exit Fractions, %	Monosaccharides after hydrolysis	Appearance of fractions
water soluble polysaccharides	4.48±0.05	glucose, xylose, ramnose	Crystalline powder, pale pink, with a characteristic odor, sour taste, soluble in water
pectin substances	5.12±0.07	glucose, galacturonic acid,	Crystalline powder of pink color, sweet taste, odorless, soluble in water

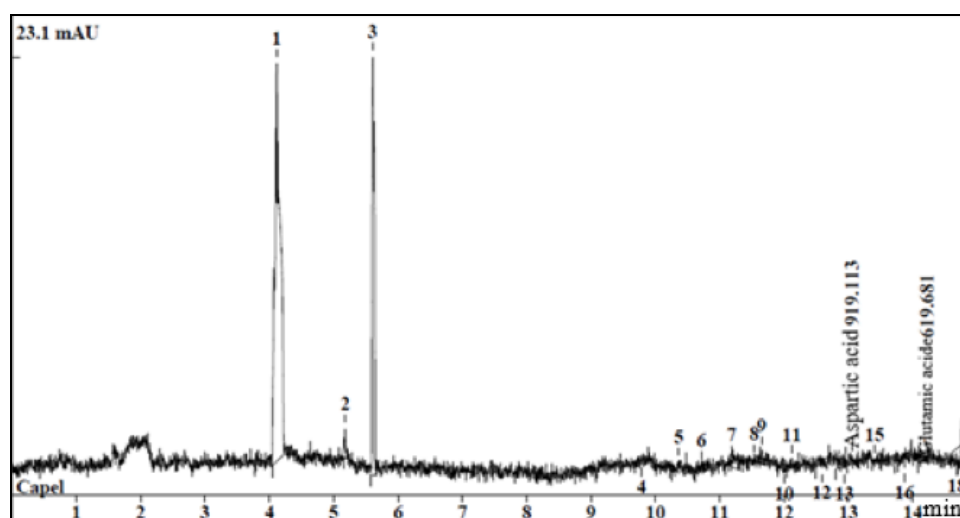
		xylose, ramnose	
hemicellulose A	3.45±0.05	glucose, xylose, ramnose	Dark brown powder, odorless, sour taste, insoluble in water
hemicellulose B	2.96±0.04		

**Table 6:** The content of free functional groups in pectin substances from *Gaillardia pulchella* inflorescences

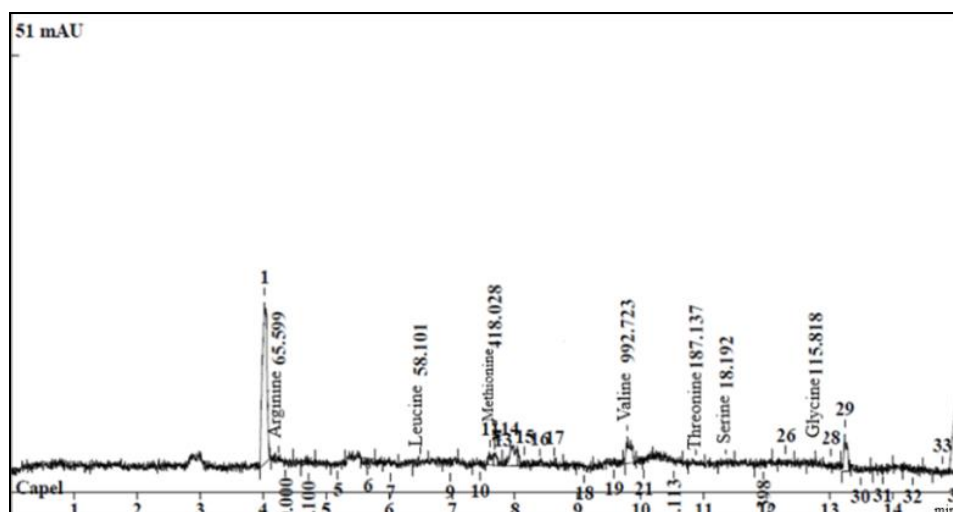
Name of indicator	Designation	Functional groups (%)
Free carboxyl groups	K <sub>c</sub>	20.61
Methoxylated groups	K <sub>M</sub>	1.35
The total number of carboxyl groups	K <sub>o</sub>	21.96
Methoxylated groups	-OCH <sub>3</sub>	0.95
Degree of esterification	λ	6.15

**Table 7:** Qualitative determination of amino acids

Coefficient of mobility of extract from <i>Gaillardia pulchella</i> inflorescences obtained by extraction with purified water	Coefficient of mobility		
	α-glycine	Glutamic acid	Valine
Solvent system: n-butanol - acetic acid - water (4: 1: 3)			
0.36±0.02	0.35±0.02		
0.50±0.02		0.48±0.02	
0.56±0.03			0.54±0.03
Solvent system: acetone - water (3: 2)			
0.46±0.02	0.45±0.02		
0.52±0.02		0.51±0.02	
0.65±0.03			0.64±0.03



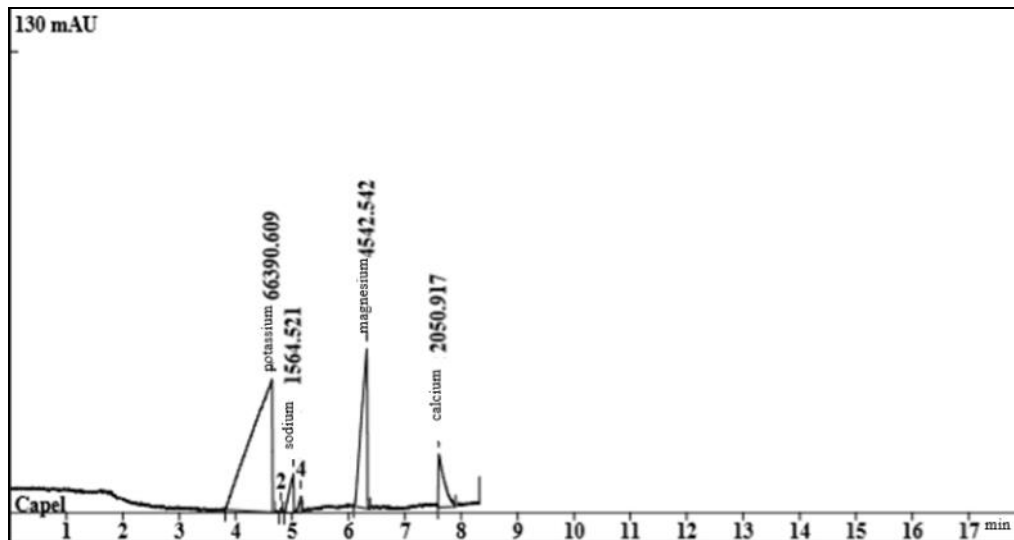
**Fig 4:** Electrophoregram of aspartic and glutamic acid in extract from the inflorescences *Gaillardia pulchella* obtained by extraction with purified water



**Fig 5:** Electrophoregram of free amino acids in the extraction of inflorescences *Gaillardia pulchella* obtained by extraction with purified water

**Table 8:** Free amino acids in extract from the inflorescences of *Gaillardia pulchella*

Amino acids	Content, mg/kg
Arginine	65,6
$\beta$ -phenylalanine	65,1
Leucine	58,1
Methionine	418,0
Valine	992,7
Proline	475,1
Threonine	187,1
Serine	18,2
$\alpha$ -alanine	16,4
Glycine	115,8
Glutamic acid	619,7
Aspartic acid	919,1

**Fig 5:** Electrophoregram of macro-and microelements**Table 9:** The content of micro and macro elements in the extraction of from inflorescences *Gaillardia pulchella* obtained by extraction with ethyl alcohol 70%

Parameter	Content, mg/kg
Potassium	66390
Sodium	1564
Magnesium	4542
Calcium	2050
Phosphorus	370
Iron	186
Manganese	17,9
Copper	47,5
Zinc	7,7
Molybdenum	0,04

**Table 10:** The results of a qualitative analysis for the presence of tannins in the water extraction of inflorescences *Gaillardia pulchella*

Reagent	Analytic effect
1% solution of Jacques	Black and green coloring (condensed tannins)
1% gelatin solution	Turbidity appears, disappearing from excess reagent
1% solution of quinine hydrochloride	Amorphous precipitate
1% antipyrine solution	Amorphous precipitate
10% CH <sub>3</sub> COOH solution, 10% solution (CH <sub>3</sub> COO) <sub>2</sub> Pb	Sediment

Black-green staining of water extracts of inflorescences *Gaillardia pulchella* in reaction with a 1% solution of iron-ammonium alum indicates the presence of condensed tannins. When a 1% solution of gelatin was added to the analyzed extracts, turbidity appeared, disappearing from an excess of reagent; with a 1% solution of quinine hydrochloride, an amorphous precipitate appeared; with a 1% solution of antipyrine - an amorphous precipitate [9, 26, 27].

#### 4. Conclusion

In the course of this study, flavonoids, polysaccharides, amino acids and tannins of the inflorescences *Gaillardia pulchella* Foug. were evaluated. In extract of *Gaillardia*

*pulchella* inflorescences obtained by extraction with 70% ethyl alcohol, the content of antioxidants turned out to be maximum.

Gravimetrically, it was found that the highest content of water-soluble polysaccharides in the meal of beautiful *Gaillardia pulchella* is 5.12%. The studied pectin substances are characterized by a low degree of etherification ( $\lambda$ ), which may indicate their high complexing ability.

The sum of flavonoids in the inflorescences *Gaillardia pulchella* is  $0.32 \pm 0.01\%$ , in terms of luteolin. The sum of antacians in the inflorescences *Gaillardia pulchella* was  $3.41 \pm 0.0355\%$ .

Based on the qualitative reactions performed, the aqueous extracts of the analyzed objects contain hydrolyzable and condensed tannins. The optimal method for the quantitative determination of tannins has been established. This is a permanganometric method in combination with the precipitation of tannins with gelatin.

In water extracts from *Gaillardia pulchella* inflorescences, the amino acids identified are aspartic and glutamic acids,  $\alpha$ -alanine,  $\alpha$ -proline, methionine and valine.

### Conflicts of interest

The authors do not declare a conflict of interest

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