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A.K. Sarkytbayeva[1*], S.A. Kubentayev[2], M.S. Kurmanbayeva[1], G.S. Aidarkhanova[3], Zh.B. Iskakova[4], M.Zh. Zhumagul[1, 2], A.B. Kusmangazinov[1]

[1]*Al-Farabi Kazakh National University, Almaty, Kazakhstan;*
[2]*Astana Botanical Garden, Astana, Kazakhstan;*
[3]*S. Seifullin Kazakh Agrotechnical University, Astana, Kazakhstan*
[4]*Research Institute of New Chemical Technologies, L.N. Gumilyov Eurasian National University, Astana, Kazakhstan*
**Corresponding author: sarkytbaeva.aisulu@gmail.com*

Paeonia anomala (*Paeoniaceae*) component composition and antioxidant, antiradical and cytotoxic activity

Paeonia anomala L. is a valuable medicinal plant in the world. The aim of this study was the study of composition of the plant *P. anomala* and its antioxidant, antiradical and cytotoxic activities. The underground part of *P. anomala* was extracted with ethanol and the composition was determined using gas chromatography with mass spectrometric detection (Agilent 7890A/5975C). Methyl salicylate (32.07 %), sucrose (11.23 %), benzoic acid (9.12 %), cyclopropyl carbine (7.7 %), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (4.77 %), benzoic acid, 2,4-dihydroxy-3,5,6-trimethyl-, methyl ester (4.48 %), are the main components. The ethyl acetate extract of the underground part (root) of *P. anomala* at concentrations of 0.25 and 0.5 mg/ml has a low content, and at concentrations of 0.75 and 1 mg/ml, an average antioxidant activity compared to the antioxidant activity of ascorbic and gallic acids. The ethanol extract of the underground part of *P. anomala* at concentrations of 0.25 and 0.5 mg/ml has an average, and at concentrations of 0.75 and 1 mg/ml the highest antioxidant activity compared to the antioxidant activity comparators. Also, the ethanol extract of the underground part *P. anomala* has the highest antiradical activity in all concentrations compared to the antiradical activity of gallic acid. Ethanol and ethyl acetate extracts of the underground part of *P. anomala* in all concentrations do not show cytotoxicity.

Keywords: Paeonia anomala, antioxidant activity, ethyl acetate extract, antiradical activity, concentration, components, ethanol extract, cytotoxic activity.

Introduction

Since herbal medicine is becoming increasingly popular, it is critical to meet the needs of both consumers and manufacturers in terms of high-quality herbal raw materials [1].

Paeonia (the only genus of *Paeoniaceae*) has 52 accepted members worldwide (36 species, 15 subspecies, and 1 variety), and is primarily found in temperate Asia, southern Europe, and western North America [2]. *P. anomala* is found from central China to Russia's Kola Peninsula, as well as northeast Kazakhstan and northern Mongolia [4]. Many species in this genus roots or root bark are commonly used as traditional medicines to treat a variety of diseases [3]. In Mongolian traditional medicine, *P. anomala* (*Paeoniaceae*) is used to treat exhaustion, bleeding, nocturnal enuresis, indigestion, blood clotting, respiratory tract disorders, abdominal pain, kidney disorders, bladder inflammation, and gynaecological diseases [5]. Furthermore, the seeds of some *Paeonia* species contain high levels of unsaturated fatty acids and are a good source of edible oil. Modern pharmacological research has shown that compounds and extracts obtained from the plants in this genus exhibit an extensive range of biological activities, including antioxidant, anti-inflammatory, antitumour, hepatoprotective, cardiovascular protective, and neuroprotective effects. It is one of the most important crude drugs in traditional European and Asian medicine, including Russia, Mongolia, and China, where it is used as an anti-inflammatory, analgesic, and sedative agent [6]. These species' various plant parts (roots, root bark, flowers, leaves, seeds, and whole plants) have been widely used to treat a variety of diseases, including female genital diseases (dysmenorrhea and amenorrhea), aches (head, stomach, eyes, and waist), neurological diseases (spasm and epilepsy), infectious diseases (carbuncles), urinary system diseases (hematuria, blood dysentery), inflammation (otitis media, append). The most commonly reported parts to be used are the roots and root bark. The potential medicinal value of the Paeonia genus is currently gaining attention [2]. The peony root, also known as “Baishao”, “Chishao”, and “Danpi,” has been used to treat dementia and blood stagnation syndrome, as well as an antihyperglycemic, analgesic, anti-inflammatory [7] and antispasmodic agent [8, 9]. The peony is an important ornamental flower that is widely planted in China,

Japan, Europe, and the United States due to its high ornamental value [10]. Currently, plant roots are used as medicines in pharmaceuticals. For example, there is a drug called "Tinctura *Paeoniae anomalae*" which is used to treat vegetative-vascular disorders, sleep disorders, and increased nervous excitation, and is manufactured in accordance with the instructions of the Republic of Belarus Ministry of Health [11, 12].

The goal of the study was to study the composition of the plant *P. anomala* and its antioxidant, antiradical and cytotoxic activity.

Experimental

Plant Material. The part (aerial and underground) of the plant *P. anomala* was collected in August 2019 from the mountain Eastern Kalba, 10 km southeast of the village. Asubulak territory of the Republic of Kazakhstan. Plot coordinates: 49º31'38" N, 83º05'16" E D., height — 1196 m above sea level m. The total projective cover is 100 %. The area of the site occupied by the species is more than 3 hectares.

Analysis methods. Gas chromatography with mass spectrometric detection (Agilent 7890A/5975C). Sample volume 1 μl sample injection temperature 250 °C without splitting. Separation was carried out using a chromatographic capillary column DB-35 MS with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 μm at a constant carrier gas (helium) velocity of 1 ml/min. The chromatography temperature is programmed from 40 °C (hold 0 min) with a heating rate of 10 °C/min to 100 °C (hold 2 min), then at a heating rate of 5 °C/min to 280 °C (hold 10 min), solvent delay 10 min analysis time 54 min. Detection is carried out in the SCAN mode m/z 34–750. Agilent MSD ChemStation software was used to control the gas chromatography system, register and process the obtained results and data. Data processing included the determination of the retention time, peak areas, as well as the processing of spectral information obtained using a mass spectrometric detector. To interpret the obtained mass spectra, the Viley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550 thousand).

Antioxidant activity determination using the FRAP method. To 0.1 ml of the investigated substances in the concentration range of 0.25; 0.5; 0.75; 1.0 mg/ml is added 0.25 ml of 0.2 M phosphate buffer (pH=6.6) and 0.25 ml of 1 % solution of potassium hexacyano ferrate (III). The reaction mixture is incubated for 20 min. At 50 °C, the reaction is stopped by adding 0.25 ml of 10 % trichloroacetic acid solution. The mixture is centrifuged for 10 minutes (3000 rpm). The top layer of 0.5 ml is mixed with 0.5 ml of distilled water and 0.1 ml of 0.1 % $FeCl_3$. The optical density is measured at 700 nm. The antioxidant activity (AOA) of the samples was compared with the AOA of ascorbic and gallic acids.

The dilution was carried out at the rate of 1 mg of the substance per 1 ml of the solvent. Each sample was tested in three parallel experiments. Carried out at a temperature of 20±2 °C, natural light period.

Antiradical activity determination using the DPPH method. To determine the inhibition of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) to 0.1 ml of an alcoholic solution of the studied solutions in the concentration range of 0.1; 0.25; 0.5; 0.75 and 1.0 mg/ml were added 3 ml of $6 \cdot 10^{-5}$ M radical solution. The test tubes were in a rack wrapped in black polyethylene. After vigorous stirring, the solutions remained in the dark for 30 minutes; then, optical densities were measured at 520 nm. The values of the antiradical activity (ARA) of the studied objects were determined by the formula:

$$ARA\ (\%) = A_0 - A_t / A_0 * 100,$$

where A_0 is the optical density of the control sample; A_t is the optical density of the working sample.

Determination of cytotoxic activity. To determine the cytotoxic activity, marine crustaceans *Artemia salina* were taken. This technique is based on establishing the difference between the number of dead *Artemia* larvae in the analyzed sample (experiment) and water that does not contain toxic substances (control). The criterion for acute lethal toxicity of a solution of a substance is the death of 50 % or more of the larvae in the experiment compared to the control.

The dilution was carried out at the rate of 1 mg of the substance per 1 ml of the solvent. Each sample was tested in three parallel experiments. Carried out at a temperature of 20±2 °C, natural light period. The salinity of the control artificial water is 8.0–8.5 (pH). During biotesting, *Artemia* larvae were under the age of 1 day. The stocking density of larvae is 20–40 specimens per tube.

Results and Discussion

The compositions of *P. anomala* in ethanol extract from the aerial part were analyzed by GC-MS (Fig. 1). 52 main compounds were detected on a chromatogram with mass spectrometric detection (Agilent 7890A/5975C). The main components are: methyl salicylate (32.07 %), sucrose (11.23 %), benzoic acid (9.12 %), cyclopropyl carbine (7.7 %), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (4.77 %), benzoic acid, 2,4-

dihydroxy-3,5,6-trimethyl-, methyl ester (4.48 %) as the major constituents. These results indicated that the differences in the profiles of the species are primarily qualitative (Table 1).

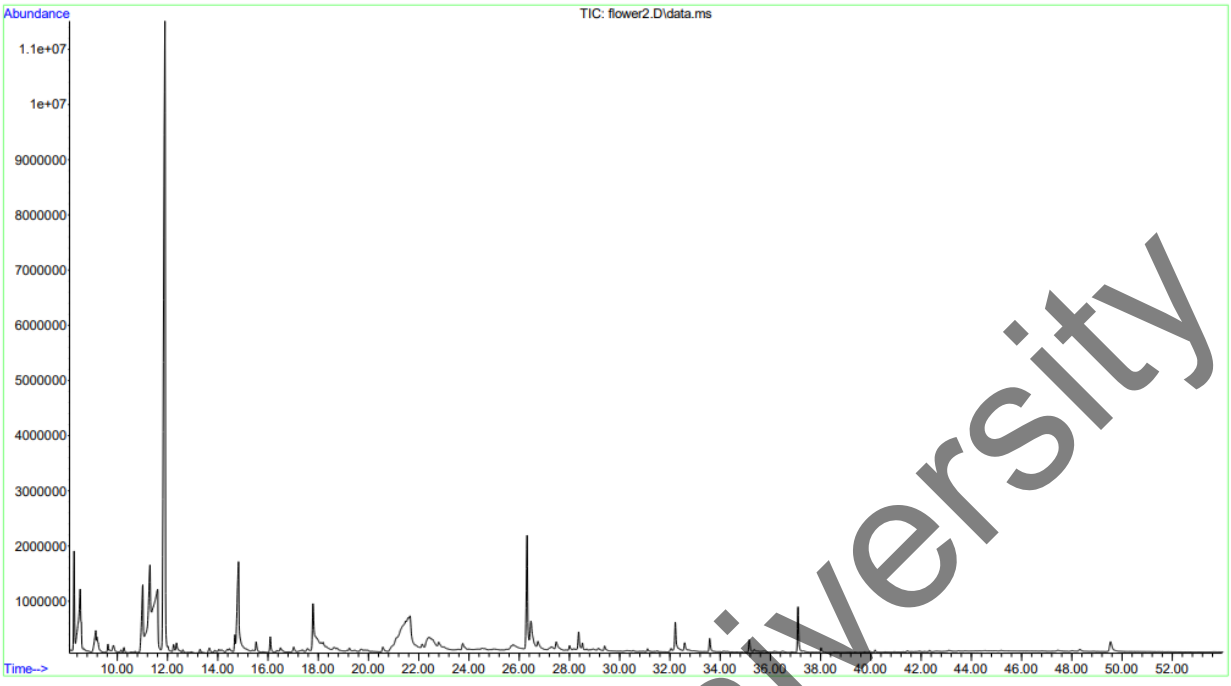

Figure 1. Chromatogram of ethanol extract from the underground part of *P. anomala*

Table 1

Component composition of the *P. anomala* (underground part)

№	Retention time, min	Component	Identification probability, %	Content, %
1	2	3	4	5
1	8,28	Benzaldehyde, 2-hydroxy-	93	2.71
2	8,52	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	73	4.77
3	9,14	D-Alanine? N-propargyloxycarbonyl-, isohexyl ester	76	2.01
4	11,01	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	87	3.66
5	11,30	Cyclopropyl carbinol	63	7.70
6	11,60	Benzoic acid	93	9.12
7	11,90	Methyl salicylate	95	32.07
8	12,36	2-Ethoxybenzyl alcohol	73	0.50
9	13,67	Benzoic acid, 2-hydroxy-, ethyl ester	73	0.30
10	13,88	2,4 (3H, 5H)-Furandione, 3,5-dimethyl-	64	0.17
11	14,04	Thymol	69	0.21
12	14,46	5-Hydroxymethyldihydrofuran-2-one	74	0.27
13	14,69	1,2,3,4,5-Tetroxane, 3,3,6,6-tetramethyl-	70	0.50
14	14,83	5-Hydroxymethylfurfural	92	6.08
15	15,53	2-Methoxy-4-vinylphenol	84	0.34
16	17,02	Benzoyl bromide	71	0.37
17	20,57	2,4-Octadienoic acid, 3-methyl-, methyl ester	65	0.40
18	21,66	Sucrose	74	11.23
19	22,13	B-D-Glucopyranose, 4-O-β-D- galactopyranosyl-	62	0.15
20	22,40	β-D-Glucopyranose, 1,6-anhydro-	79	1.86
21	22,79	α-D-Glucopyranose, 4-O-β-D- galactopyranosyl-	67	0.38
22	23,75	β-d-Lyxofuranoside, O-nonyl-	66	0.58
23	26,32	Benzoic acid, 2,4-dihydroxy-3,5,6-trimethyl-, methyl ester	77	4.48
24	26,47	3-Ethoxy-4-methoxybenzaldehyde	69	1.98

Continuation of Table 1

1	2	3	4	5
25	26,75	3,4-Methylenedioxypropiophenone	68	0.49
26	27,48	Benzo[b]thiophene-2-carboxaldehyde, 7-methyl-,	74	0.67
27	28,37	Hexadecanoic acid	86	0.74
28	28,52	Hexadecanoic acid, ethyl ester	82	0.26
29	29,40	Benzoic acid, 2-hydroxy-, phenylmethyl ester	82	0.28
30	31,11	Methyl 9-cis, 11-trans- octadecadienoate	71	0.15
31	32,04	Oleic Acid	60	0.16
32	32,22	9, 12,-Octadecadienoic acid, ethyl ester	87	1.47
33	32,59	9,12,15-Octadecatrienoic acid	79	0.32
34	33,59	11-Oxatricyclo [5.3.0.1 (2,6)] undecan-4-one, 3 endo-5-endo-dimethyl-9-isopropyl	65	0.52
35	37,10	Benzoic acid, 4-acetylbenzyl ester	84	1.69
36	38,02	Methyl 2α, 3β-dihydroxyolean-12-en-28-oate	66	0.19
37	40,17	Triphenyl phosphate	77	0.11
38	41,45	Phosphoric acid, 4-methylphenyl diphenyl ester	65	0.12
39	48,31	β-Sitosterol acetate	62	0.23
40	49,55	Vitamin E	87	0.77

According to the study findings, the main components were discovered in the underground part. These components are critical for the parts they discovered. For example, Esters are also essential compounds for plants. They impart many distinct odors and flavours to plants. Esters play an important role in plant cell division and serve a structural function in plant cell membranes [13–14]. Methyl salicylate (wintergreen oil) is widely available over-the-counter in a variety of liniments, ointments, lotions, and medicated oils for the relief of musculoskeletal aches and pains [15]. Sucrose esters are widely used in the food and cosmetic industries, and there is growing interest in their use in various pharmaceutical fields [16]. Plant benzoic acids (BAs) serve as building blocks or essential structural elements for a wide range of primary and specialized metabolites, including plant hormones, cofactors, defense compounds and pollinator and seed disperser attractants [17].

The FRAP (Ferric Reducing Antioxidant Power assay) method is based on antioxidants reducing Fe^{3+} ions to Fe^{2+}. A reduction reaction with antioxidants $K_3[Fe(CN)_6]$ is used, which is accompanied by the formation of yellow-colored $K_4[Fe(CN)_6]$. The measurements are based on antioxidants' ability to suppress the oxidative effect of reaction particles produced in the reaction mixture. Ascorbic and gallic acids were used as reference drugs. Concentrations of 0.25, 0.5, 0.75, and 1 mg/ml were used to test the samples (Table 2, Fig. 2).

Table 2

Change in the optical density of solutions depending on the concentration of working solutions

№	Samples	Concentration optical density (mg/ml)			
		0,25	0,5	0,75	1,0
11	Ascorbic acid (AA)	1,5539	1,5928	1,6775	1,7738
32	Gallic acid (GA)	1,4859	1,5423	1,6867	1,7665
33	Ethyl acetate extract *P. anomala* (P.anom-1)	0,4626	0,6705	0,8864	1,1411
44	Ethanol extract *P. anomala* (P.anom-2)	0,8981	1,1134	1,5621	1,7094

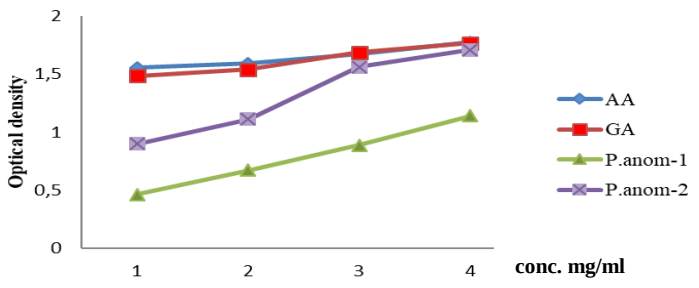

Figure 2. Influence of the concentration of substances on the change in antioxidant activity

Based on the analysis of the data in Table 2 and Figure 2, it can be seen that the ethyl acetate extract of the underground part of *P. anomala* (P.anom-1) at concentrations of 0.25 and 0.5 mg / ml has a low content, and at concentrations of 0.75 and 1 mg/ml average antioxidant activity compared to AOA of ascorbic and gallic acids. Ethanol extract of the underground part of *P. anomala* (P.anom-2) at concentrations of 0.25 and 0.5 mg/ml has an average, and at concentrations of 0.75 and 1 mg/ml, it has a higher antioxidant activity compared to AOA reference drugs. E. Enkhtuya et al. investigated antioxidant activity. *P. anomala*, a 50 % ethanol extract of peony leaf, demonstrated remarkable DPPH radical scavenging activity, ABTS radical cations, and superoxide anions, as well as significant ferric reducing antioxidant power. The extract also had a high concentration of phenolics. The ethanol extract was successively separated using various polar organic solvents to form hexane, diethyl ether, ethyl acetate, butanol and water fractions. The antioxidant capacity of ethyl acetate and diethyl ether fractions is higher than that of the original ethanol extract [2].

The concentration-dependent optical density of the studied solutions (antiradical activity) was measured on a spectrophotometer at a wavelength of 520 nm. Gallic acid was used as a reference drug. Samples were tested with concentrations of 0.1; 0.25; 0.5; 0.75 and 1 mg/ml (Table 3).

Table 3

Change in the optical density of the studied solutions with a change in concentration

№	Test substances	Optical density values by concentration (mg/ml)				
		0,1	0,25	0,5	0,75	1,0
1	Gallic acid (GA)	0.1396	0.1357	0.1257	0.1242	0.1155
2	Ethyl acetate extract of the underground part of *P. anomala* (P.anom-1)	0.7113	0.6790	0.6638	0.6240	0.6020
3	Ethanol extract of the underground part of *P. anomala* (P.anom-2)	0.1734	0.1702	0.1617	0.1571	0.1541

The antiradical activity of the studied solutions was compared with the antiradical activity of gallic acid (GA). The values of the studied extracts of the antiradical effect, calculated by the formula, are given in Table 4 and Figure 3.

Table 4

Antiradical activity (%) of extracts at different concentrations

№	Test substances	Extract concentration (mg/ml)				
1	Gallic acid (GA)	80.35	80.90	82.3	82.51	83.74
2	Ethyl acetate extract of the underground part of *P. anomala* (P.anom-1)	16.52	20.30	22.09	26.76	29.34
3	Ethanol extract of the underground part of *P. anomala* (P.anom-2)	75.60	76.04	77.24	77.90	78.31

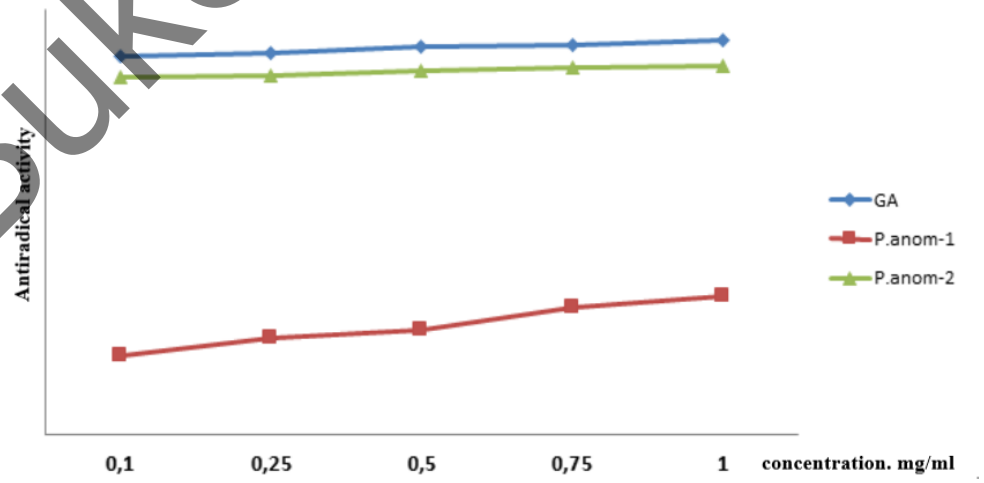

Figure 3. Dynamics of antiradical activity with a change in the concentration of substances

Based on the analysis of the data in the table and graph, it can be seen that the ethyl acetate extract of the underground part of *P. anomala* (P.anom-1) has a low content, and the ethanol extract of the underground part of *P. anomala* (P.anom-2) has the highest antiradical activity in all concentrations compared with the antiradical activity of gallic acid.

Cytotoxic activity. Cytotoxic activity was studied by the method of survival of marine crustaceans Artemia salina. The flask was filled with artificial sea water and *Artemia salina* eggs were added. They were kept for 3 days with a soft air supply until the crustaceans hatched from the eggs.

Paclitaxel-Teva was used as a reference drug. Samples were tested at concentrations of 10, 5 and 1 mg/ml. The results of studies of cytotoxic activity are shown in Table 5.

Table 5

Results of the study of cytotoxic activity

Test substances	Concentrations, mg/ml	Number of larvae in control		The number of larvae in the sample			% surviving larvae in control	% surviving larvae in the sample	Mortality, A, %	Presence of neurotoxicity, %
		survivor	lost	survivor	lost	parallel				
Paclitaxel-Teva	10	22	1	0	22	0	96	0	96	0
	5	22	1	1	25	0	96	4	92	0
	1	22	1	9	18	0	96	33	63	0
Ethanol extract of *P. anomala*	10	22	1	18	5	0	96	78	18	0
	5	22	1	24	3	0	96	89	7	0
	1	22	1	25	2	0	96	92	4	0
Ethyl acetate extract of *P. anomala*	10	22	1	26	4	0	96	86	10	0
	5	22	1	25	2	0	96	92	4	0
	1	22	1	24	1	0	96	96	0	0

As a result of the study of cytotoxic activity, it was found that ethanol and ethyl acetate extracts of the underground part of *P. anomala* do not exhibit cytotoxicity at all concentrations. Comparator drug Paclitaxel-Teva in relation to marine crustaceans *Artemia salina* in all concentrations exhibits cytotoxicity — the mortality of larvae is 63–96 %.

Conclusion

Eventually, the chemical composition of *P. anomala* aerial parts was determined. As a result of this research, 52 plant components were quantified. Because of the presence of these bioactive components, the plant extract could have a variety of properties. The ethyl acetate extract of the underground part of *P. anomala* (P.anom-1) at concentrations of 0.25 and 0.5 mg/ml has a low content, and at concentrations of 0.75 and 1 mg/ml, an average antioxidant activity compared to ascorbic and gallic acids. Ethanol extract of the underground part of *P. anomala* (P.anom-2) at concentrations of 0.25 and 0.5 mg/ml has an average, and at concentrations of 0.75 and 1 mg/ml, it has a higher antioxidant activity compared to AOA reference drugs. When compared to the antiradical activity of gallic acid, the ethyl acetate extract of the underground part of *P. anomala* (P.anom-1) has a low content, and the ethanol extract of the underground part of *P. anomala* (P.anom-2) has a higher antiradical activity in all concentrations. Furthermore, no cytotoxicity is observed at any concentration.

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А.К. Саркытбаева, С.А. Кубентаев, М.С. Курманбаева, Г.С. Айдарханова,
Ж.Б. Искакова, М.Ж. Жумагул, А.Б. Кусмангазинов

Paeonia anomala (Paeoniaceae) компоненттік құрамы және оксидантқақарсы, радикалғақарсы, цитотоксикалық белсенділіктері

Paeonia anomala L. — дүниежүзіндегі бағалы дәрілік өсімдік. Зерттеудің мақсаты — *P. anomala* өсімдігінің құрамы мен оның оксидантқақарсы, радикалғақарсы және цитотоксикалық белсенділігін табу. *P. anomala* жерасты бөлігі этанолмен бөлініп алынды және құрамы масс-спектрометриялық детектирлеудің (Agilent 7890A/5975C) газ хроматографиясының көмегімен анықталды. Циклогексанон, 5-метил-2-(1-метилетилиден)- (25,2 %), 1-ментон (15,3 %), 2-метокси-4-винилфенол (6,7 %), бицикло[3.1.1]гепт-3-en -2-бір, 4,6,6-триметил-, (1S)-(4,7 %), фенол, 2-метил-5-(1-метилэтил)-(4,2 %) — негізгі компоненттер. *P. anomala* жерасты бөлігінің этилацетаты (тамырының) сығындысы 0,25 және 0,5 мг/мл концентрацияда төмен, ал 0,75 және 1 мг/мл концентрацияда аскорбин және галл қышқылдарының оксидантқақарсы белсенділігімен салыстырғанда орташа оксидантқа қарсы белсенділікке ие. *P. anomala* жерасты бөлігінің спирттік сығындысы 0,25 және 0,5 мг/мл концентрацияда орташа, ал 0,75 және 1 мг/мл концентрацияда антиоксиданттық белсенділігі бар салыстыру препараттарымен салыстырғанда ең жоғары антиоксиданттық белсенділікке ие. Сондай-ақ, *P. anomala* жерасты бөлігінің этанол сығындысы галл қышқылының антирадикал белсенділігімен салыстырғанда барлық концентрацияларда ең жоғары радикалғақарсы белсенділікке ие. *P. anomala* жерасты бөлігінің этанол және этилацетаты сығындылары барлық концентрацияларда цитоуыттылық белсенділік көрсетпейді.

Кілт сөздер: Paeonia anomala, оксидантқақарсы белсенділік, этилацетат сығындысы, радикалғақарсы белсенділік, концентрация, компоненттер, этанол сығындысы, цитотоксикалық белсенділік.

А.К. Саркытбаева, С.А. Кубентаев, М.С. Курманбаева, Г.С. Айдарханова,
Ж.Б. Искакова, М.Ж. Жумагул, А.Б. Кусмангазинов

Компонентный состав и антиоксидантная, антирадикальная, цитотоксическая активность *Paeonia anomala (Paeoniaceae)*

Paeonia anomala L. — ценное лекарственное растение в мире. Целью настоящего исследования было изучение состава растения *P. anomala* и его антиоксидантной, антирадикальной и цитотоксической активности. Надземную часть *P. anomala* экстрагировали этанолом и определяли состав с помощью газовой хроматографии с масс-спектрометрическим детектированием (Agilent 7890A/5975C). Циклогексанон, 5-метил-2-(1-метилэтилиден)- (25,2 %), 1-ментон (15,3 %), 2-метокси-4-винилфенол (6,7 %), бицикло[3.1.1]гепт-3-ен-2-он, 4,6,6-триметил-, (1S)-(4,7 %), фенол, 2-метил-5-(1-метилэтил)- (4,2 %) являются основными компонентами. Этилацетатный экстракт подземной части (корень) *P. anomala* в концентрациях 0,25 и 0,5 мг/мл обладает низкой, а в концентрациях 0,75 и 1 мг/мл — средней антиоксидантной активностью по сравнению с АОА аскорбиновой и галловой кислот. Спиртовой экстракт подземной части *P. anomala* в концентрациях 0,25 и 0,5 мг/мл обладает средней, а в концентрациях 0,75 и 1 мг/мл — наибольшей антиоксидантной активностью по сравнению с препаратами сравнения антиоксидантной активностью. Также этанольный экстракт подземной части *P. anomala* имеет наибольшую антирадикальную активность во всех концентрациях по сравнению с антирадикальной активностью галловой кислоты. Этанольный и этилацетатный экстракты подземной части *P. anomala* во всех концентрациях не проявляют цитотоксической активности.

Ключевые слова: Paeonia anomala, антиоксидантная активность, этилацетатный экстракт, антирадикальная активность, концентрация, компоненты, этанольный экстракт, цитотоксическая активность.