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QUALITATIVE CHARACTERISTICS OF PHOSPHATE BUFFER EXTRACTS FROM WOODY AND SHRUB PLANTS OF THE MOUNTAIN RANGES OF EASTERN AND SOUTHEASTERN KAZAKHSTAN

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Abstract. The article investigates the qualitative characteristics of phosphate-buffered extracts obtained from the leaves and fruits of woody and shrub species growing across various mountain ranges in Eastern and Southeastern Kazakhstan. Samples of *Rosa acicularis*, *Rosa albertii*, *Rosa tarbagataica*, *Prunus armeniaca*, and *Prunus bucharica* were used as research materials. Extraction was performed using a 50 mM phosphate buffer at pH 7.0. The obtained extracts were evaluated for colour, turbidity, sediment formation, and stability. The results revealed distinct qualitative differences among plant species and the examined plant organs. Leaf extracts were generally characterised by dark green or brownish-green colouration and higher turbidity. In contrast, fruit extracts exhibited a yellowish or light-brown colour and were more transparent. Samples belonging to the genus *Rosa* showed higher turbidity and more pronounced sediment formation, whereas fruit extracts of the genus *Prunus* were characterised by greater stability and lower turbidity. The findings indicate that the phosphate-buffered system is a suitable medium for obtaining plant extracts and allows a comparative assessment of the qualitative characteristics of extracts from different plant species. The results may serve as baseline data for planning subsequent biochemical analyses and studies.

Keywords: 50 mM phosphate buffer, plant tissues, qualitative characteristics of extracts, turbidity, precipitate formation, water-soluble proteins, *Rosa*, *Prunus*.

КАЧЕСТВЕННЫЕ ОСОБЕННОСТИ ЭКСТРАКТОВ ДРЕВЕСНО-КУСТАРНИКОВЫХ РАСТЕНИЙ ГОРНЫХ ХРЕБТОВ ВОСТОЧНОГО И ЮГО-ВОСТОЧНОГО КАЗАХСТАНА В ФОСФАТНО-БУФЕРНОЙ СИСТЕМЕ

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Аннотация. В статье исследованы качественные особенности фосфатно-буферных экстрактов, полученных из листьев и плодов древесно-кустарниковых растений, произрастающих в различных горных хребтах Восточного и Юго-Восточного Казахстана. В качестве объектов исследования использованы образцы видов *Rosa acicularis*, *Rosa albertii*, *Rosa tarbagataica*, *Prunus armeniaca* и *Prunus bucharica*. Экстракцию проводили с использованием 50 мМ фосфатно-буферного раствора с pH 7,0. Оценивались цвет, степень мутности, образование осадка и стабильность полученных экстрактов. Результаты исследования показали наличие выраженных качественных различий между видами растений и исследуемыми органами. Экстракты, полученные из листьев, в основном характеризовались тёмно-зелёной или буровато-зелёной окраской и более высокой степенью мутности. Экстракты плодов имели желтоватую или светло-коричневую окраску и отличались сравнительно большей прозрачностью. Для образцов рода *Rosa* были характерны более высокая мутность и интенсивное образование осадка, тогда как плодовые экстракты рода *Prunus* отличались относительной стабильностью и меньшей степенью мутности. Полученные результаты свидетельствуют о том, что фосфатно-буферная система является подходящей средой для получения растительных экстрактов и позволяет проводить сравнительную оценку качественных особенностей экстрактов различных видов растений. Результаты исследования могут быть использованы в качестве исходных данных при планировании последующих биохимических исследований.

Ключевые слова: 50 мМ фосфатный буфер, растительные ткани, качественные характеристики экстрактов, мутность, образование осадка, водорастворимые белки, *Rosa*, *Prunus*.

ШЫҒЫС ЖӘНЕ ОҢТҮСТІК-ШЫҒЫС ҚАЗАҚСТАН ТАУ ЖОТАЛАРЫНЫҢ АҒАШ-БҰТА ӨСІМДІКТЕРІ ЭКСТРАКТТАРЫНЫҢ ФОСФАТТЫ-БУФЕРЛІК ЖҮЙЕДЕГІ САПАЛЫҚ ЕРЕКШЕЛІКТЕРІ

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Аңдатпа. Мақалада Шығыс және Оңтүстік-Шығыс Қазақстанның әртүрлі тау жоталарында таралған ағаш-бұта өсімдіктерінің жапырақтары мен жемістерінен алынған фосфатты-буферлік экстракттардың сапалық ерекшеліктері зерттелді. Зерттеу нысандары ретінде *Rosa acicularis*, *Rosa albertii*, *Rosa tarbagataica*, *Prunus armeniaca* және *Prunus bucharica* түрлерінің үлгілері пайдаланылды. Экстракция pH 7,0 деңгейіндегі 50 мМ фосфатты-буферлік ерітінді көмегімен жүргізілді. Алынған экстракттардың түсі, лайлану дәрежесі, тұнба түзілуі және тұрақтылығы бағаланды. Зерттеу нәтижелері өсімдік түрлері мен зерттелген мүшелер арасында айқын сапалық айырмашылықтардың бар екенін көрсетті. Жапырақтардан алынған экстракттар негізінен қою жасыл немесе қоңыр-жасыл түсті болып, лайлану деңгейінің жоғары болуымен сипатталды. Ал жемістерден алынған экстракттар сарғыш немесе ашық қоңыр түсті болып, салыстырмалы түрде мөлдірлігі жоғары болды. *Rosa* туысына жататын үлгілерде лайлану мен тұнба түзілу деңгейі жоғары байқалса, *Prunus* туысының жеміс экстракттары салыстырмалы тұрақтылығымен және төмен лайлануымен ерекшеленді. Алынған нәтижелер фосфатты-буферлік жүйенің өсімдік ұлпаларынан экстракттар алуға жарамды орта екенін және әртүрлі өсімдік түрлерінің экстракттарының сапалық ерекшеліктерін салыстырмалы бағалауға мүмкіндік беретінін көрсетті. Зерттеу нәтижелері кейінгі биохимиялық талдауларды жоспарлау үшін бастапқы деректер ретінде пайдаланылуына мүмкіндік береді.

Түйін сөздер: 50 мМ фосфатты буфер, өсімдік ұлпалары, экстракттардың сапалық сипаттамалары, лайлану, тұнба түзілуі, *Rosa*, *Prunus*.

Introduction. Tree and shrub plants distributed in the mountainous regions of eastern and South-Eastern Kazakhstan are an important natural source of biologically active substances, including *Rosa* spp., *Prunus* spp., *Berberis* spp., and *Crataegus* spp. The leaves and fruits of other species are widely used in folk medicine, pharmaceuticals and the food industry. These plants accumulate proteins, vitamins, mineral elements, phenolic compounds, and flavonoids, which confer high biological and antioxidant activity (Tayjanov et.al., 2017; Ситпаева, Г. Т., et.al., 2025.).

In the extraction of proteins from plant materials, phenolic compounds, tannins, pigments, and other secondary metabolites significantly impact the study's results. These compounds can bind to proteins, leading to extract turbidity, precipitate formation, and decreased accuracy of subsequent biochemical analyses (Ryabushkina et.al., 2008). Therefore, the preliminary assessment of the qualitative properties of extracts is considered an important stage in protein research.

Various buffer systems are used to isolate water-soluble proteins from plant tissues. Among them, phosphate-buffered solutions stand out for maintaining pH stability, protecting the natural structure of proteins, and being compatible with many biochemical methods (Wink, M. 2010).

The purpose of this work is to study the qualitative characteristics of phosphate buffer extracts obtained from the leaves and fruits of Woody and shrub plants distributed across the mountain ranges of Eastern and South-Eastern Kazakhstan, to conduct a comparative assessment of their physico-chemical characteristics, and to determine their suitability for protein extraction.

Materials and methods. The object of the research was the leaves and fruits of tree and shrub plants distributed in the mountainous regions of Eastern and South-Eastern Kazakhstan. The research area includes plant species growing in various natural and climatic zones of the Altai, Saur, Tarbagatai, Zhetysu Alatau, Zailiyskiy Alatau and Tien Shan mountain systems. Plant samples selected for the study were collected from different altitudes and sites with varying climatic conditions and soil characteristics.

The leaves and fruits of the species *Rosa acicularis, Rosa albertii, Rosa tarbagataica, Prunus armeniaca* and *Prunus bucharica* were used as research objects. The full list of studied samples is presented in Table 1.

Table 1. Description of samples of Woody and shrub plants collected from the mountain ranges of Eastern and South-Eastern Kazakhstan

No.	Latin name of plant species	Studied plant organ	Mountain ranges	Sampling site	Mass
1	*Rosa acicularis*	leaf	Sunkar Hill	Ridder	0.1
2	*Rosa acicularis*	fruit	Sunkar Hill	Ridder	0.1
3	*Rosa albertii*	leaf	Saur-Tarbagatai mountain range	Tarbagatai	0.1
4	*Rosa albertii*	fruit	Saur-Tarbagatai mountain range	Tarbagatai	0.1
5	*Rosa tarbagataica*	leaf	Katon-karagay mountain range	Sarymsakty Ridge, Katon-Karagai	0.1
6	*Rosa tarbagataica*	fruit	Katon-karagay mountain range	Sarymsakty Ridge, Katon-Karagai	0.1
7	*Rosa tarbagataica*	leaf	Saur-Tarbagatai mountain range	Tarbagatai	0.1
8	*Rosa tarbagataica*	fruit	Saur-Tarbagatai mountain range	Tarbagatai	0.1
9	*Prunus armeniaca*	leaf	Ketpen (Uzynkara) Ridge	Karadala Sumbe	0.1
10	*Prunus armeniaca*	fruit	Ketpen (Uzynkara) Ridge	Karadala Sumbe	0.1
11	*Prunus bucharica*	leaf	Control sample	Uzbekistan 2025	0.1
12	*Prunus bucharica*	fruit	Control sample	Uzbekistan 2025	0.1

Plant materials were collected during the active phase of the growing season and delivered to the laboratory in paper bags after mechanical impurities were removed. In laboratory conditions, the samples were matched according to morphological and taxonomic characteristics. Only healthy, undamaged tissue was used in the study. Before analysis, the samples were stored at +4 °C (Agati, G., et.al., 2012: Orazov, A. et.al., 2022).

For the extraction of water-soluble compounds from plant tissues, a 50 mM phosphate buffer solution at pH 7.0 was used. The buffer was prepared from sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$). To prepare 100 ml of the buffer, 0.39 g of $NaH_2PO_4 \cdot 2H_2O$ and 0.45 g of $Na_2HPO_4 \cdot 12H_2O$ were dissolved in 80–90 ml of distilled water. The pH was monitored using an electronic pH meter. The final volume

was then brought to 100 ml with distilled water (Sambrook, J., et.al., 2001; Green, M. et.al., 2021).

Before extraction, 0.1 g of material was accurately measured from each plant sample. Samples were placed in polypropylene test tubes with a volume of 1.5 ml, and a phosphate-buffer solution of 1.0 ml was added on top. The ratio of plant material to buffer was kept at 1:10 for all samples (Scopes, R. K. 1993).

Homogenization was performed using a laboratory homogeniser. Each sample was processed for 90 seconds, then homogenised again for 90 seconds after a 30-second break. Such processing ensured the destruction of plant cells and increased the efficiency of the transition of water-soluble compounds to a buffer medium (Mm, B. 1976).

After homogenization, the suspensions were centrifuged at 4 °C for 10 minutes at 12,000 rpm. As a result of centrifugation, cellular waste settled to the sediment, leaving a supernatant in the upper layer. For subsequent analyses, the supernatant was used (Kruger, N. J., et.al., 2009).

The qualitative characteristics of the obtained phosphate-buffer extracts were evaluated. During the evaluation, the colour, transparency, degree of turbidity, sediment formation and stability of the extracts during storage were determined. Turbidity was visually assessed and classified into clear, weak, medium, and dark categories; the presence or absence of sediment indicated sediment formation. The suitability of extracts for subsequent spectrophotometric analyses was also evaluated. The results obtained enabled the relative characterisation of the qualitative features of phosphate-buffered extracts from different plant species (Green, M. R., et.al., 2019).

Results and discussion. The qualitative characteristics of phosphate-buffer extracts from the leaves and fruits of Woody and shrub plants distributed in the mountain ranges of eastern and South-Eastern Kazakhstan were studied. During the study, the colour, degree of turbidity, sediment formation, and external stability of the extracts were evaluated. These indicators were considered important features characterising the overall quality of extracts from plant tissues.

The degree of turbidity of extracts was assessed on a 4 – point scale: 0 points – transparent, 1 point – weak turbidity, 2 points – medium turbidity, 3 points-thick turbidity. The intensity of sediment formation was also determined on a 4 – point scale: 0 points – no sediment, 1 point – weak sediment, 2 points – medium sediment, 3 points-clear sediment. Such an assessment enabled a relative assessment of the qualitative differences between samples (Wilson, K., et.al., 2018).

The leaves and fruits of the species *Rosa acicularis, Rosa albertii, Rosa tarbagataica, Prunus armeniaca* and *Prunus bucharica* were used as research objects. In general, extracts from leaf samples were dark green or brownish-green in colour, while extracts from fruit samples were characterised by orange, light brown or pale yellow. Such differences may be associated with the features of plant organ pigment systems (Wilson, K., et.al., 2018).

According to the study, the Rosa acicularis leaf extract was deep dark green in colour and exhibited a high level of turbidity (3 points). At the same time, a pronounced precipitate was observed at the bottom of the extract (3 points). The extract from the fruits turned out to be dark brown in colour and appeared in a muddy State (2 points). Sediment formation was also observed (2 points). Such features of extracts may be associated with the presence of phenolic compounds and other secondary metabolites in plant tissues described in literary sources (Wilson, K., et.al., 2018).

Extracts from the leaves of *Rosa albertii* were dark green in colour and “high turbidity” (3 points). Extracts from fruits have a yellowish-brown colour and an average turbidity (2 points). During storage, some samples showed increased turbidity. According to the literature, such changes are likely associated with the oxidation of phenolic compounds (Hames, B. D. 1998).

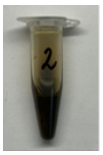
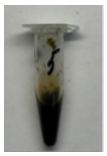
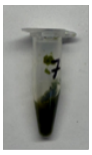
In *Rosa* tarbagataica samples, the colour intensity of the extracts was higher. Extracts from leaf samples were deep dark green in colour and distinguished by a high level of turbidity (3 points). In some samples, a mixed precipitate was observed. The colour of the extracts from the fruits was orange-yellow-brown or whitish-Orange. The turbidity rate ranged from weak to moderate (1-2 points). According to the literature, such features may be associated with the amount of phenolic compounds, flavonoids, and other secondary metabolites present in the plant (Scopes, R. K. 1993; Taiz, L., et.al., 2014).

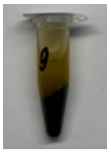
The *Prunus armeniaca* leaf extract was greenish-brown and moderately turbid (2 points). The

extract from the fruit samples was light brown, weakly muddy (1 point). The relative transparency of the extract may be related to the constant storage of the water-soluble components in it in a buffer medium (Dai, J., et.al., 2010).

The *Prunus bucharica* leaf extract was dark brown-green and highly turbid (3 points). And the extract from the fruits was pale yellow, weakly muddy (1 point). Among the samples studied, this extract showed one of the highest levels of transparency. According to literary data, such properties may be associated with the predominance of water-soluble compounds in fruit tissues. Significant differences were found in the colour of the extracts, the degree of turbidity and the features of sediment formation. The results are shown in Table №2.

Table 2. Qualitative characteristics of phosphate-buffer extracts from Woody and shrub plants of the mountain ranges of Eastern and South-Eastern Kazakhstan

No.	Sample code	Studied plant organ	Color	Turbidity	Precipitate	Note	Photo
1	*Rosa acicularis*	leaf	Deep dark green	high turbidity (3 points)	3 points	With a high chlorophyll content, cell residues are observed; among the extracts with a high turbidity rate are.	
2	*Rosa acicularis*	fruit	Black-brown	Muddy (2 points)	2 points	Dark colour extract; turbidity is observed due to the high content of phenolic compounds.	
3	*Rosa albertii*	leaf	Dark green	high turbidity (3 points)	3 points	A high concentration of pigments is observed; the level of turbidity is high among the studied leaf samples.	
4	*Rosa albertii*	fruit	Yellowish-brown	moderate turbidity (2 points)	2 points	The extraction is relatively stable, the sediment amount is small, and the transparency is moderate.	
5	*Rosa tarbagataica*	leaf	Deep dark green	high turbidity (3 points)	3 points	With a high chlorophyll content, large particles were observed; mixed precipitate formation was detected, and one sample had the highest turbidity.	
6	*Rosa tarbagataica*	fruit	Orange-yellow-brown	moderate turbidity (2 points)	2 points	The content of pigments and phenolic compounds is high; stability is average.	
7	*Rosa tarbagataica*	leaf	Dark green	Muddy (2 points)	2 points	An inhomogeneous suspension is observed; the formation of a mixed precipitate indicates that solid particles have been preserved during extraction.	
8	*Rosa tarbagataica*	fruit	Light orange	low turbidity (1 point)	1 point	The extract is relatively clean, with high transparency and low precipitate content.	

No.	Sample code	Studied plant organ	Color	Turbidity	Precipitate	Note	Photo
9	*Prunus armeniaca*	leaf	Green-brown	Muddy (2 points)	2 points	Dark colour extraction; plant residues are found; turbidity is moderate.	
10	*Prunus armeniaca*	fruit	Light brown	low turbidity (1 point)	1 point	The extract is uniform and relatively stable; it is among the most transparent samples.	
11	*Prunus bucharica*	leaf	Dark brown-green	high turbidity (3 points)	2 points	The content of chlorophyll and phenolic compounds is high; although the turbidity is high, the extract is uniform.	
12	*Prunus bucharica*	fruit	Pale yellow	low turbidity (1 point)	1 point	One of the most transparent and most stable extracts among the samples studied; sediment formation was observed at the lowest level.	

The study found that leaf extracts were much darker and muddier than fruit extracts. This phenomenon is explained by the high accumulation of photosynthetic pigments, structural proteins, and phenolic compounds in leaf tissues, as reported in the literature (Haslam, E. 1989). In addition, the interaction of phenolic compounds with proteins and other macromolecules can contribute to the turbidity of extracts (Kumar, S., et.al., 2013).

In samples from the genus *Rosa*, high turbidity and sedimentation were observed. Especially in the specimens of *Rosa albertii* and *Rosa tarbagataica*, these indicators were clearly manifested. According to the literature, plants growing at high altitudes may have a high content of phenolic compounds and antioxidants that perform protective functions, which is likely to affect the colour and transparency of the extracts (Buchanan, B. et.al., 2015).

In fruit specimens of the genus *Prunus*, the level of turbidity was low, and the relative stability of extracts was observed. Such a feature may be due to the high proportion of water-soluble carbohydrates and other low-molecular compounds in their chemical composition.

In general, the study found that the qualitative indicators of plant extracts depend on the plant type and the organ studied. While specimens of the genus Rosa showed high turbidity and sedimentation, fruit specimens of the genus *Prunus* were relatively transparent and stable. The extracted extracts can be used as a starting material for subsequent biochemical studies, including analyses such as determining the amount of Total Protein. However, because protein concentration was not determined within the scope of this study, no clear conclusions can be drawn about the effectiveness of protein extraction using the phosphate-buffered system.

Conclusion. The qualitative features of phosphate-buffer extracts obtained from the leaves and fruits of Woody and shrub plants distributed in various mountain ranges of eastern and South-Eastern Kazakhstan have been identified. The leaves and fruits of the species *Rosa acicularis, Rosa albertii, Rosa tarbagataica, Prunus armeniaca* and *Prunus bucharica* were used as research objects. For extraction, a phosphate-buffered solution at 50 mM, pH 7.0, was used, and the colour, transparency, degree of turbidity, and sedimentation features of the extracts were evaluated.

The study's results showed clear qualitative differences between plant species and their respective members. Extracts from leaf samples were mostly dark green or brown in colour, while extracts from fruit samples were orange, brown, or light in colour.

In most *Rosa* samples, the extracts were highly turbid, and sedimentation was observed. Especially in samples from the species *Rosa albertii* and *Rosa tarbagataica*, dark colour, high

turbidity, and high sedimentation intensity were observed. Such features indicate that the listed species may contain a relatively large number of phenolic compounds, flavonoids and other secondary metabolites. Also, these substances are more likely to affect the physical properties of extracts, reducing their transparency.

The fruit extracts from plants in the genus *Prunus* were relatively transparent and stable. Especially in extracts from the fruits of *Prunus armeniaca* and *Prunus bucharica*, turbidity rates were low, and sediment formation was less noticeable.

The results obtained showed that the phosphate-buffer solution is a suitable medium for extracting from the studied plant materials. The buffer used enabled preservation of the main qualitative features of the samples during extraction and allowed comparison of the properties of extracts from different plant species.

The identified qualitative differences indirectly characterise the biochemical composition of plant species and indicate their potential to be rich in biologically active substances. These data complement the study of the biochemical potential of natural plant resources in Kazakhstan and serve as a basis for assessing the potential of various plant species for biotechnological, pharmacological, and food applications.

The conducted study enabled assessment of the qualitative characteristics of plant extracts, and the results obtained can serve as a basis for determining protein amounts using the Bradford method in subsequent studies.

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