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ANTIOXIDANT ACTIVITY AND BIOCHEMICAL COMPOSITION OF *Morus alba* AND *Morus nigra* SPECIES

F.I. ISLAMOVA[✉], G.K. RADJABOV, A.M. MUSAEV

Mountain Botanical Garden, Dagestan Federal Research Center RAS, 45, ul. Gadgieva, Makhachkala, Republic Dagestan, 367000 Russia, e-mail fatimaisl@mail.ru (✉ corresponding author), chemfarm@mail.ru, musaev-58@list.ru

ORCID:

Islamova F.I. orcid.org/0000-0003-4804-3168

Musaev A.M. orcid.org/0000-0001-6692-8571

Radjabov G.K. orcid.org/0000-0001-9263-5684

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Abstract

Mulberry (*Morus* L.), a woody plant popular in Russia is of great economic benefits. On the territory of the Dagestan Republic, there are two *Morus* species, the *Morus alba* L. and *Morus nigra* L. The mulberry fruits, from leaf, bark and root extracts have powerful antioxidant, anti-inflammatory, antimicrobial properties and analgesic effects. In this paper, we revealed for the first time a high antioxidant status for two species, *M. alba* and *M. nigra* (cv. Hartut) growing in the Republic of Dagestan. Biochemical analysis revealed variability of the antioxidant level in the mulberry fruit pomace, leaves, bark, and roots. It was revealed that fruit pomace contains the required amount of mono-, disaccharides and bioactive compounds (e.g., water-soluble vitamins, organic acids) and has a rich mineral composition. The aim of the research was to assess the biological activity and biochemical composition of the fruit pomace, leaves, bark, and roots of mulberry plants grown in Dagestan. Plant material was sampled in the second decade of June 2020 (OOO Nizam, the suburbs of the city of Makhachkala, settlement Leninkent, Republic of Dagestan). The total content of antioxidants was measured amperometrically (a Tsvet Yauza 01-AA device, OAO NPO Khimavtomatika, Russia). The concentrations of water-soluble vitamins, organic acids and sugars were measured by capillary electrophoresis (a Kapel-105M system, OOO Lumex-marketing, Russia). Micro- and macro elements were quantified by atomic absorption spectrometry using ordinary acetylene-air flame atomization (a SavantAAΣ atomic absorption spectrometer, GBC Scientific Equipment Pty Ltd., Australia). The research results showed a high antioxidant status of fruit pomace, especially for white-fruited *M. alba* (400.73 mg/g) and *M. nigra* cv. Hartu (363.77 mg/g). A high level of antioxidants also occurred in leaves, bark, and roots. The antioxidant concentration in the leaves and roots of black-fruited form of *M. alba* was the highest, 44.56 mg/g and 71.79 mg/g, respectively. *M. nigra* plants have the highest amount of antioxidants in the bark, 36.33 mg/g. The quantitative determination revealed a higher content of vitamins C (ascorbic acid) and B₉ (folic acid) in mulberry fruit pomace, and the white-fruited form of *M. alba* (31.4 and 5.2 mg%) and *M. nigra* cv. Hartut (29.0 and 6.0 mg%) were most prominent. Qualitative and quantitative analysis revealed 10 chemical elements in plants, of which five (Na, K, Ca, Mg, Fe) were of leading importance. Potassium in a larger amount (342.6 mg%) occurred in *M. alba* (black-fruited form), a high concentration of Ca (50.6 mg%) was characteristic of *M. alba* (pink-fruited form). *M. nigra* stood out by the accumulation of Mg (54.6 mg%). *M. alba* (black-fruited) contained the largest amount of Na (16.5 mg%). *M. alba* (pink-fruited) was distinguished by the content of Fe (3.1 mg%). As to organic acids of *M. alba* and *M. nigra*, in the samples, we revealed only malic and citric acids. In all samples, the content of citric acid was 1.5-2 times higher than that of malic acid, except for the pink-fruited *M. alba*. Of mono- and disaccharides (fructose, glucose, sucrose), glucose dominated quantitatively in all samples, especially in black-fruited *M. alba* (10.40 %). Our findings indicate the high biological value of the *M. alba* and *M. nigra* species and their high potential for the development of therapeutic and prophylactic food products, bioactive additives, etc. Therefore, more phytochemical studies are necessary to search and reproduce the most valuable forms for practical use and improvement.

Keywords: antioxidants, *Morus alba*, *Morus nigra*, cv. Hartut, macro elements, microelements, vitamins, organic acids, sugars

Oxidative stress disrupts the body natural antioxidant system, increases risks for various dangerous diseases, and reduces life expectancy [1-4]. It is known

that antioxidants from their natural sources (medicinal plants, fruits, vegetables, seeds, berries) and derived functional products can neutralize the excess of free radicals. Currently, plant materials containing antioxidants which have a mild effect on the body are increasingly used for preventive purposes [5-7].

Antioxidants slow down free radical reactions, protect cell membranes and DNA from destruction. In medicine, the use of plant materials is largely due to the content of bioflavonoids which have anti-inflammatory, antiviral, antimutagenic, antitumor and hepatoprotective effects [8-11]. Traditionally, for the design of a healthy diet, fruit and berry raw materials are used, which are characterized by high biological and pharmacological activity [12, 13].

In recent years, interest in the phytochemical characteristics and pharmacological value of the mulberry culture (*Morus* L.) has increased [14, 15]. It is known that the genus *Morus* consists of 10-16 species distributed in the temperate and subtropical zones of Asia, Africa, and North America. Mulberry is a woody plant of great economic importance. Fruits of the genus *Morus* have powerful antioxidant properties, extracts from the bark and roots have an analgesic effect [16, 17]. Extracts from the leaves and flowers of the white mulberry have anti-tuberculosis activity and immunomodulating properties [18, 19]. The roots, bark and fruits of *Morus* plants contain a powerful antioxidant resveratrol which normalizes cellular metabolism and enhances oxygen transport, regulates fat metabolism in the liver, strengthens the walls of blood vessels, improves blood rheology, and also has anti-allergic, radioprotective, anti-inflammatory, antimicrobial properties [20-23]. Due to powerful biological potential, *Morus* plants are attractive as a source of raw material for functional nutrition products.

In the present work, we have for the first time established the high antioxidant status of fruit pomace in two *Morus* species, *M. alba* and *M. nigra* (cv. Hartut), growing in the Republic of Dagestan. The data obtained assess the degree of variability in the amount of antioxidants not only in fruit pomace, but also in other parts of the plant (leaves, bark, and roots). We have found that fruit pomace contains the required amount of mono-, disaccharides and bioactive substances (vitamins, organic acids), and also has a rich mineral composition.

Our goal was to study the antioxidant activity and biochemical composition of fruit pomace, leaves, bark, and roots in two species, *Morus alba* and *M. nigra* (cv. Hartut) under the conditions of the Republic of Dagestan.

Materials and methods. Mulberry pomace and parts of plants (leaves, bark, and roots) of *M. alba* (white-fruited form), *M. alba* (dark-fruited form), *M. alba* (pink-fruited form), and *M. nigra* (cv. Hartut) were used in the study.

Samples were collected in 2020 during mass fruit ripening in the second decade of June in a private nursery OOO Nizam located in the suburbs of Makhachkala (Leninkent village, Republic of Dagestan). The trees of the same age (27 years old) were standing separately. The site is irrigated, the soils are chestnut, loamy, 2-3% humus.

The total content of antioxidants in fruit pomace and plant parts was measured amperometrically (a Tsvet Yauza 01-AA device, OAO NPO Khimavtomatika, Russia), based on the electric current in the electrochemical cell, which occurred when a certain potential was applied to the electrode. For a calibration graph, in order to exclude random results, solutions of gallic acid trihydrate (purity > 98.5%) (Sigma-Aldrich, China) were prepared with a mass concentration of 0.2; 0.4; 2.0; 4.0 mg/l to be consecutively measured in five repetitions of which three estimates were used in statistical processing. Orthophosphoric acid (Komponent-reaktiv, Russia) with a molar fraction of 0.0022 mol/dm³ was an eluent. Using calibration, the signals of the studied extract were compared to the signals of the reference sample, the gallic acid. The total concentration of antioxidants

was expressed in mg/g [24].

The concentration of water-soluble vitamins (Methodology M 04-72-2011, <https://www.lumex.ru/methodics/20ARU03.13.03-1.pdf>), organic acids and sugars in fruit pomace was estimated based on the separation of the ionic forms of the analyzed components by the capillary electrophoresis (a Kapel-105M system, OOO Lyumex-marketing, Russia). The amount of water-soluble vitamins was determined at an electric field voltage of 25 kV and $\lambda = 200$ nm, organic acids at -20 kV and $\lambda = 254$ nm (Method M 04-47-2012, <https://www.lumex.ru/methodics/20ARU03.01.09-1.pdf>), sugar content at 25 kV and $\lambda = 254$ nm (Methodology M 04-69-2011, <https://www.lumex.ru/methodics/20ARU03.15.03-1.pdf>).

The mineral composition was assayed by the atomic absorption method with atomization in an acetylene-air flame (a SavantAA Σ atomic absorption spectrometer, GBC Scientific Equipment Pty Ltd., Australia). The concentration of macro-, micro- and ultramicroelements was expressed in mg% (MU 01-19/47-11).

The obtained data were statistically processed using the Microsoft Excel package and the Statistika 5.5 program (StatSoft, Inc., USA). The mean values (M) and relative standard deviation (RSD) values were determined, which in our case did not exceed 0.1% (with an allowable 5%). Based on the RSD, the error of the arithmetic mean SEM was calculated (the sample error estimate of the mean is RSD/\sqrt{n}). The processing was performed by the one-way analysis of variance. Significance between sample means was assessed by Fisher's F -test. The probability of confirming the null hypothesis (p -level) was also calculated.

Results. In our experiment, we evaluated plants of the *M. alba* species of folk selection with different fruit colors. This is especially important because *M. nigra* plants are rare in Dagestan both in cultivation and as a wild form and are commonly confused with dark-fruited forms of *M. alba*. As a model for the *M. nigra*, we chose the ancient Iranian black mulberry cv. Hartut, the original area of which is the Derbent District of the Republic of Dagestan, where immigrants from Northern Iran live.

1. Total content of antioxidants (mg/g) in fruit pomace and in parts of plants of genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Fruit pomace	Plant part		
		leaves	bark	roots
<i>Morus alba</i> (white-fruited form)	400,73 \pm 0,051	21,29 \pm 0,053	15,00 \pm 0,002	59,15 \pm 0,060
<i>Morus alba</i> (dark-fruited form)	266,00 \pm 0,135	44,56 \pm 0,012	16,00 \pm 0,052	71,79 \pm 0,014
<i>Morus alba</i> (pink-fruited form)	257,07 \pm 0,076	5,04 \pm 0,003	19,18 \pm 0,024	17,51 \pm 0,005
<i>Morus nigra</i> (cv. Hartut)	363,77 \pm 0,014	6,71 \pm 0,041	36,33 \pm 0,033	30,18 \pm 0,062

The antioxidant concentration in the fruit pomace of the studied samples was high and ranged from 257.07-400.73 mg/g, with the white-fruited form of *M. alba* being especially prominent (Table 1). It should be noted that in the work of researchers from Pakistan, the total antioxidant activity for *M. nigra* was 1.19-1.25 mmol trolox/g, for *M. alba* 0.75-0.78 mmol trolox/g [25]. According to the content of antioxidants in the leaves and roots, the dark-fruited form of *M. alba* was distinguished, 44.56 mg/g and 71.79 mg/g, respectively, which was statistically ($p \leq 0.01$) higher than in other samples. For antioxidants in the bark, *M. nigra* prevailed with the value of 36.33 mg/g. According to the literature, all parts of *Morus* plants are used in medicine, and extracts obtained from them have antioxidant, antiinflammatory, antibacterial, and antiviral properties [26, 27].

The one-way analysis of variance shows high reliability of differences between the samples in the total content of antioxidants (Table 2).

It should be noted that the accumulation of antioxidants was higher in the samples of *Morus* compared to spicy-aromatic and essential oil plants [28]. Thus, a route survey of wild-growing populations of cumin (*Carum carvi*) revealed that

its seeds contain from 0.76 to 1.47 mg/g of antioxidants. In another ecogeographic examinations, the total amount of antioxidants in coriander seeds was 1.2-3.7 mg/g, depending on the variety, and in dill seeds 6.5-12 mg [28, 29]. In addition, the total content of antioxidants in the samples we studied exceeded those in foods, juices, teas and coffees [30].

2. One-way analysis of variance of total content of antioxidants in fruit pomace and in parts of plants of genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Plant part	SS	df _A	mSA	SSE	df _E	MSE	F	p
Pomace	46104.26	3	15368.09	1.346067	8	0.168258	91336	0.000000
Leaves	557.05	3	185.68	0.020362	8	0.002545	72952	0.000000
Bark	896.07	3	298.69	0.000874	8	0.000109	2733050	0.000000
Tooyes	5680.34	3	1893.45	0.491826	8	0.061478	30799	0.000000

Note. SS — sum of squares, df_A — degree of freedom, mSA — mean square, SSE — sum of squares of error, df_E — degree of freedom of error, MSE — mean square error, F — Fisher's test, p — significance level for the null hypothesis of no difference between averages.

3. Accumulation of water-soluble vitamins in fruit pomace in plants of genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Content of vitamin, mg%						
	B ₁	B ₂	B ₆	C	B ₃	PP	B ₉
<i>Morus alba</i> (white-fruited form)	0.04±0.000	0.02±0.000	0.03±0.000	31.4±0.00	0.5±0.00	0.8±0.00	5.2±0.01
<i>Morus alba</i> (dark-fruited form)	0.03±0.001	0.04±0.000	0.07±0.002	27.8±0.00	0.4±0.00	0.7±0.00	4.7±0.01
<i>Morus alba</i> (pink-fruited form)	0.02±0.000	0.04±0.000	0.06±0.001	25.6±0.00	0.3±0.00	0.8±0.00	4.4±0.00
<i>Morus nigra</i> (cv. Hartut)	0.04±0.002	0.03±0.000	0.05±0.001	29.0±0.00	0.1±0.00	0.7±0.00	6.0±0.00

All *Morus* samples had a high content of vitamins C (ascorbic acid) and B₉ (folic acid) (Table 3). The highest accumulation of vitamins C and B₉ occurred in the white-fruited form of *M. alba* (31.4 and 5.2 mg%, respectively) and *M. nigra* (29.0 and 6.0 mg%). It is known that two phenolic groups in the structure of the ascorbic acid molecule allows it to participate in redox processes as a hydrogen donor and acceptor. Vitamin C reduces the amount of hydroxyl and peroxide radicals, restoring the active form of vitamin E and glutathione [31].

The total content of antioxidants in fruit pomace we obtained for plants of the genus *Morus* cannot be explained only by a high amount of ascorbic acid. Additional studies are needed on the accumulation of strong antioxidants in this crop capable of generating strong amperometric signal. In our opinion, these may include the hydrocarbon stilbene and its derivatives. Chemical compounds based on folic acid (folates, vitamin B₉) are involved in methylation of proteins, hormones, lipids, neurotransmitters, enzymes and other essential components of metabolism, nucleotide synthesis and DNA replication, cell division and normal growth of the body [32-3].

Qualitative and quantitative analysis of *Morus* raw materials revealed 10 chemical elements (Table 4) of which five were of leading importance (Na, K, Ca, Mg, and Fe). A comparative analysis showed that the content of chemical elements in *Morus* samples varied within different limits. Potassium, which regulates the state of the cytoplasm of plant cells and accelerates photosynthetic phosphorylation, was found in a greater amount (342.6 mg%) in the dark-fruited form of *M. alba*. A high concentration of Ca, which is part of the plant cell wall, was found in pink-fruited form of *M. alba*, 50.6 mg%. According to the accumulation of Mg, a cofactor of many enzymes, *M. nigra* stood out with 54.6 mg%. The amount of Na, which regulates the transport of carbohydrates in the plant, was higher in dark-fruited form of *M. alba*, 16.5 mg%. The content of Fe involved in the creation of chlorophyll and the process of plant respiration, was the highest in pink-fruited form of *M. alba*, 3.1 mg%. The sufficient amount of essential minerals

in *Morus* culture can be considered as a bioavailable complex that plays a physiological role in the functioning of many body systems [36, 37].

4. Mineral composition of fruit pomace in plans of genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Element	Concentration, mg%			
	<i>Morus alba</i> (white-fruited form)	<i>Morus alba</i> (dark-fruited form)	<i>Morus alba</i> (pink-fruited form)	<i>Morus nigra</i> (cv. Hartut)
Macroelements				
Na	15.2±0.03	16.5±0.07	12.8±0.06	14.0±0.06
K	286.3±0.14	342.6±0.49	328.6±0.34	309.8±0.20
Ca	42.2±0.20	45.1±0.17	50.6±0.06	28.7±0.08
Mg	48.3±0.05	45.9±0.29	42.4±0.17	54.6±0.25
Microelements				
Fw	2.5±0.02	2.6±0.00	3.1±0.00	2.4±0.03
Cu	0.02±0.000	0.05±0.001	0.01±0.000	0.03±0.001
Zn	0.31±0.001	0.28±0.002	0.35±0.041	0.38±0.004
Cr	0.003±0.0000	0.003±0.0000	0.005±0.0000	0.004±0.0000
Mn	0.012±0.0001	0.024±0.0000	0.010±0.0000	0.03±0.001
Al	0.32±0.002	0.41±0.003	0.35±0.002	0.44±0.005
Ni	0.01±0.000	0.02±0.000	0.01±0.000	0.02±0.001
Iodides	0.001±0.0000	0.002±0.0000	0.001±0.0000	0.002±0.0000
Ultramicroelements				
Pl	0.002±0.0000	0.002±0.0000	0.004±0.0000	0.003±0.0000

Among organic acids (Table 5), we detected only malic and citric acids. In all samples, the concentration of citric acid was 1.5-2 times higher than that of malic acid, except for the pink-fruited form of *M. alba*. The amount of free organic acids in *Morus* samples was several times higher than the requirements established by the State Pharmacopoeia of the Russian Federation (at least 2.6%). This is of importance for food biotechnology, since these acids provide optimal conditions for a full-fledged digestion and have the ability to suppress the development microorganisms due to the concentration of hydrogen ions.

5. Accumulation of organic acids and sugars in fruit pomace in genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Organic acids, mg%		Sugars, %		
	malic acids	citric acids	fructose	glucose	sucrose
<i>Morus alba</i> (white-fruited form)	10,30±0,110	54,40±0,060	4,36±0,004	7,68±0,000	0,68±0,004
<i>Morus alba</i> (dark-fruited form)	20,40±0,052	57,90±0,050	7,66±0,010	10,40±0,005	0,71±0,006
<i>Morus alba</i> (pink-fruited form)	24,76±0,083	14,40±0,030	4,80±0,001	8,21±0,003	0,70±0,001
<i>Morus nigra</i> (cv. Hartut)	12,16±0,031	21,90±0,030	4,91±0,000	8,07±0,002	0,75±0,003

According to accumulation of mono- and disaccharides (fructose, glucose and sucrose) in fruit pomace, glucose dominated quantitatively in all samples, especially in the dark-fruited form of *M. alba*, 10.40% (see Table 5). It is known that the fruits of *M. nigra* are 82.9-86.2% water and 10.9-12.7% sugars. In dried fruits, carbohydrates accounted for approx. 73.3-83.7% [38]. In our study, the required levels of mono- and disaccharides additionally makes the culture attractive.

The results of one-way analysis of variance showed (Table 6) that the differences between the studied samples in terms of water-soluble vitamins, organic acids, and sugars were highly significant.

6. One-way analysis of variance of concentration of water-soluble vitamins, organic acids and sugars in fruit pomace in genus *Morus* ($N = 3$, $M \pm SEM$; settlement Leninkent, Republic of Dagestan, 2020)

Substances	F	p
Vitamins		
B1	315	0.000000
B2	297	0.000000
B6	948	0.000000
C	150492	0.000000
B3	2576	0.000000
PP	500	0.000000
B9	4831	0.000000

			<i>Continued Table 6</i>
Organic acids	Apple acid	7309.50	0.000000
	Lemon acid	197474.10	0.000000
Sugars	Fructose	18954.63	0.000000
	Glucose	41603.08	0.000000
	Sucrose	40.17	0.000036
	Sum of sugars	52633.17	0.000000

Note. *F* — Fisher's test, *p* — significance level for the null hypothesis of no difference between averages.

It should be noted that the mulberry is a not used fruit resource. It requires additional research to develop a technology for its field harvesting and processing. It is necessary to search for the genetic resources of both species and create a collection of local assortment of cultivars to develop programs for breeding large-fruited varieties. It also requires an inventory of local methods of processing mulberry fruit products (drying, production of anhydrous syrups, juices and low-alcohol drinks, etc.) for the development of technical conditions and GOST standards.

Thus, we have established a high antioxidant status of fruit and berry raw materials of the *Morus* plants, in particular, the white-fruited form of *M. alba* (400.73 mg/g) and *M. nigra* cv. Hartut variety (363.77 mg/g), growing in the territory of the Republic of Dagestan. The high antioxidant activity of the leaves and roots of the dark-fruited form of *M. alba* and the bark of *M. nigra* have been also shown. The revealed value of fruit pomace in *M. alba* and *M. nigra* cv. Hartut is due to the accumulation of biologically active compounds (vitamins, organic acids), as well as a rich mineral composition. Our findings indicate that the *Morus* crops are valuable fruit and medicinal sources of interest for phytochemistry and the food industry.

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