EFFECTS OF ULTRASOUND ON THE EXTRACTION OF ANTIOXIDANTS FROM BEARBERRY (*ARCTOSTAPHYLOS ADANS)* LEAVES

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The effects of ultrasound (f = 60 kHz) on the quantity of biologically active substances extracted from bearberry leaves into a water:ethanol (1:1) mixture were studied, along with their antioxidant properties. The optimal conditions for extract preparation were identified: the raw material:extractant ratio and the duration of ultrasound exposure. Positive and negative aspects of the ultrasound extraction of antioxidants are discussed. The antioxidant activity of the extract prepared in optimum conditions was greater than that of a bearberry leaf extract prepared without ultrasound, increasing the resistance of sunflower oil to oxidation by factors of 2 – 2.2.

Antioxidants are currently obligatory components of many products of the food and pharmaceutical industries and are used to enrich the vitamin composition of products and to preserve the quality of easily oxidized fatty components [1]. The use of antioxidants in these industries is strictly regulated by All-Union State Standards (GOST) and Sanitary Regulations and Standards (SanPin) requirements [2, 3] which approve the use of both synthetic (butylhydroxyanisol, butylhydroxytoluene) and natural (tocopherols, quercetin) substances. There is great interest in the natural sources of a complex of natural antioxidants – plant infusions and extracts, which not only have high antioxidant activity, comparable with the efficacies of synthetic antioxidants, but whose biological activities also have a series of advantages over synthetic compounds [4].

The preparation of extracts and infusions with these properties generally requires prolonged processes, so there is interest in finding methods allowing rapid, economic, and ecologically sustainable preparation of effective antioxidants from raw materials. Studies of this type are directed at intensifying the limiting stage of the extraction process – internal molecular diffusion. This is achieved using a series of physical activators of this process: freezing, pressing, application of high pressure, and others [5]. Intensification of the extraction process can also be obtained using ultrasound. However, the high energy density and the intensities of processes occurring in an ultrasound irradiation field generate the need to investigate their chemical actions on the substances extracted. The actions of ultrasound during the extraction of antioxidants from plant raw material have yet to be identified.

The aim of the present work was to study the effects of ultrasound on the quantity of materials extracted from plants and their antioxidant activities.

METHODS

Extraction of a complex of biologically active substances (BAS) was performed using dry ground (2 mm diameter) bearberry leaves, which contain a variety of phenolic compounds [6]. The extractant was a mixture of water and ethanol (1:1) [7]. Extraction was performed at a temperature of 293°K in a glass extractor placed in a soundproofing jacket. The source of ultrasound waves was a Colibri ultrasound acoustic generator (f = 60 kHz) located at the center of the extractor. The duration of extraction was varied from 10 to 45 min. The resulting liquid extract was separated from the raw material by filtration, and was then dried in a vacuum drying chamber at 293°K to constant weight. Extracts were standardized in terms of the quantity of dry residue ($W_{\text{dry, mass\%}}$), measured gravitometrically [8]. The antioxidant activity (AOA) of the resulting dried extracts was studied by chemoluminescence (CL) in conditions of induced (azodiisobutyronitrile (AIBN), dibromoanthracene (DBA), which are

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A) H_2O —) ——) ———) H_2O^*;

H_2O —) ——) ———) $H_2O^+ + e^-$;

$H_2O^* \rightarrow H^+ + OH^-$;

$H_2O^+ \rightarrow H^+ + {}^\bullet OH$;

$e + aq \rightarrow eaq^\bullet$

B) Radial-transforming reactions:

$H^\bullet + O_2 \rightarrow HO_2^\bullet$

${}^\bullet OH + H_2 \rightarrow H^\bullet + H_2O$

B) Overall water break-up reaction:

H_2O —) ——) ———) $H^\bullet$, ${}^\bullet OH$, e_{aq}, H_2, H_2O_2

Scheme 1. Chemical conversion of water during ultrasound.

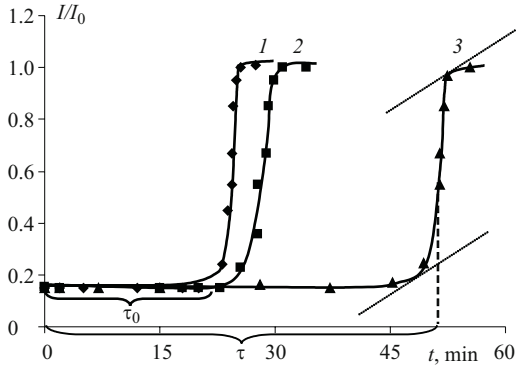

Fig. 1. Kinetic curves showing the relative chemiluminescence intensity (I/I_0) in conditions of initiated ([AIBN] = 2×10^{-2} M, [DBA] = 2×10^{-3} M, R = 343°K) oxidation of sunflower oil (*1*), with addition of water:ethanol extracts of bearberry leaves (1:25) prepared by maceration (*2*) and ultrasound maceration (*3*).

luminescence activators) oxidation of sunflower oil (GOST 1129–93) in chlorobenzene solution (1:1) at 343°K [9]. Antioxidant activity was measured in terms of τ/τ_0, where τ is the oil oxidation induction time in the presence of 0.1 mass% of extract and τ_0 is the time in the absence of extract (Fig. 1).

RESULTS AND DISCUSSION

The use of ultrasound is based on a number of physical effects occurring when waves propagate in the irradiated system. The leading role is played by cavitation and cavitation effects, which disrupt cellular structures in the outer layers of particles of the plant material. Investigation of the effects of ultrasound waves on the extraction of biologically active substances and their antioxidant properties allowed us to identify the optimum ultrasound exposure time for bearberry leaves and the corresponding raw material:extractant ratio. The results presented in Table 1 show that increases in the duration of extraction both without and with ultrasound increased the concentration of natural substances in the extract, i.e., increased the dry weight of the extract, though irradiation yielded more complete extraction of the extractable material from the raw material in a shorter period of time. This is probably associated with the intense leaching of substances from the disrupted cells due to microimpacts from imploding cavities at the surface between the extractant and the raw material, along with the appearance of pulsatile microflows propagating into the plant material along capillaries, increasing the solubility and coefficient of internal molecular diffusion of the extractable substances.

TABLE 1. Total Quantities of Extracted Substances ($W_{mass,\%}$) and AOA (τ/τ_0) in Water-Ethanol Extracts of Bearberry Leaves.

Duration of extraction, min	Raw material:extractant ratio							
	1:40		1:30		1:25		1:20	
	$W_{mass,\%}$	τ/τ_0	$W_{mass,\%}$	τ/τ_0	$W_{mass,\%}$	τ/τ_0	$W_{mass,\%}$	τ/τ_0
without ultrasound								
10	0.61	1.01	0.65	1.03	0.70	1.05	0.73	1.05
15	0.67	1.01	0.69	1.03	0.73	1.05	0.75	1.05
20	0.69	1.03	0.72	1.09	0.82	1.12	0.85	1.08
25	0.74	1.10	0.77	1.12	0.89	1.14	0.86	1.15
30	0.77	1.10	0.81	1.12	0.96	1.15	0.95	1.15
35	0.82	1.10	0.86	1.15	1.08	1.21	1.12	1.17
40	0.83	1.11	0.92	1.15	1.12	1.23	1.21	1.17
45	0.85	1.12	1.01	1.17	1.17	1.23	1.25	1.18
f = 60 kHz								
10	1.21	1.20	1.58	1.22	1.71	1.22	1.69	1.21
15	1.27	1.25	1.61	1.32	1.75	1.45	1.72	1.40
20	1.30	1.31	1.64	1.60	1.82	1.63	1.77	1.58
25	1.32	1.45	1.69	1.71	1.91	1.79	1.86	1.74
30	1.37	1.54	1.75	1.85	2.02	1.98	1.91	1.88
35	1.39	1.43	1.86	1.75	2.11	2.10	1.94	1.95
40	1.42	1.28	1.92	1.72	2.15	2.04	1.96	1.91
45	1.45	1.19	2.01	1.70	2.21	2.01	1.97	1.90

TABLE 2. Effects of Degassing of Water on Extract (1:25) AOA and the Quantity of Substances Extracted from Bearberry Leaves.

Duration of extraction, min	Water			
	Without degassing		Degassing	
	$W_{mass,\%}$	τ/τ_0	$W_{mass,\%}$	τ/τ_0
10	1.71	1.22	1.72	1.25
15	1.75	1.45	1.76	1.52
20	1.82	1.63	1.82	1.67
25	1.91	1.79	1.93	1.89
30	2.02	1.98	2.01	2.12
35	2.11	2.10	2.11	2.22
40	2.15	2.04	2.15	2.22
45	2.21	2.01	2.21	2.19

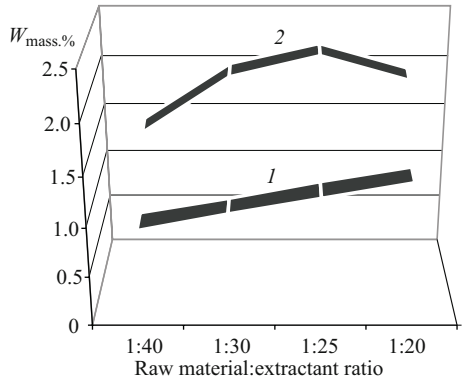

Fig. 2. Relationship between dry weight of extract and the quantity of plant raw material in the extraction system: *1*) without irradiation; *2*) with irradiation.

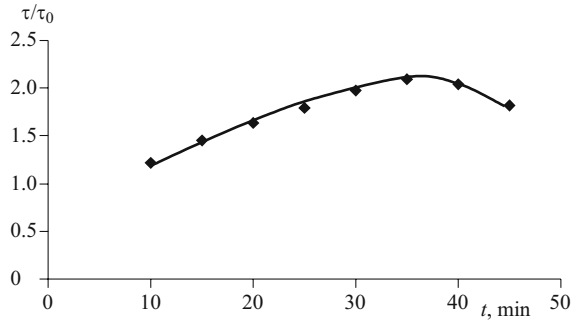

Fig. 3. Relationship between the antioxidant activity of water:ethanol (1:1) extracts of bearberry leaves (1:25) and the duration of irradiation of the system, $T = 293$°K.

The yield of biologically active substances from the plant material on exposure to ultrasound was affected by the raw material:extractant ratio. Table 1 shows that increases in the mass proportion of bearberry leaves in the system had different effects on the quantity of dry residue and its antioxidant activity. Figure 2, *a* shows that with simple maceration, increases in the quantity of material extracted were proportional to the mass of raw material used. In irradiated systems (Fig. 2*b*), there were increases in the yields of extractable substances at low (1:40) to intermediate (1:25) raw material:extractant ratios. Further increases in the ratio led to reductions in the BAS content of extracts. Decreases in substance yields were probably due to the fact that increases in the quantity of solid raw material particles in the system decrease the transmission of ultrasound energy by the solvent. This in turn leads to decreases in the number of cavities able to rupture cells in the plant material and hence to less intense leaching of substances from them.

Attention is drawn to the relationship between the antioxidant activity of the resulting extract and the duration of irradiation (Table 1, Fig. 3). Increases in the AOA of the extract for raw material:extractant ratios of 1:40 and 1:30 were seen during the first 30 min of ultrasound extraction, while at ratios of 1:25 and 1:20, the time required for extraction of antioxidants was longer (35 min). Further irradiation of the system led to decreases in extract AOA, apparently because of activation of a system of chemical changes affecting the extracted substances with antioxidant properties (phenolic compounds). The chemical actions of ultrasound are known to be based on a number of physicochemical processes occurring in cavitation cavities. Ionization of water is particularly relevant [10], along with the resulting formation of active radical particles. During extraction, natural phenols take part in the inactivation of these radicals, decreasing their quantities in the extract and reducing its AOA. The presence of dissolved gases (O_2, H_2) in water significantly expand the qualitative and quantitative composition of active particles (Scheme 1) [10], which may be reflected in the composition and quantity of phenolic compounds present in the extract. Consumption of phenols by reaction with radicals formed by ultrasound extraction in water was prevented by altering the composition of radicals by removing gases from the solvent by vacuum degassing. The results presented in Table 2 show that experiments using degassed water yielded an extract with a higher level of AOA, with virtually no change in the quantity of BAS extracted ($W_{dry,\ mass\%}$). Decreases in extract AOA were seen on prolonged ultrasound extraction, lasting more than 40 min.

Results obtained from studies of the effects of ultrasound waves ($f = 60$ kHz) on the quantity of extracted substances and their AOA indicate that the irradiation process had both positive (intensification of the release of biologically active substances) and negative (inactivation of phenols) actions on extract quality.

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