ELECTRIC-FIELD-ASSISTED EXTRACTION OF ANTIOXIDANTS FROM BEARBERRY LEAVES

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Conditions for improving the extraction of substances with antioxidant properties from bearberry (*Arctostaphylos Adams*) leaves have been studied. It is established that the natural antioxidants are most completely extracted by maceration in a constant electric field (U = 35 V, I = 250 mA) for 2 h at 303 K with stirring. The optimum extractant is 1% aqueous acetic acid with added surfactant (Tween-80, 8.8×10^{-4} M). This extract increases the stability (storage time) of sunflower oil more than four times. The proposed method of electroextraction increases the yield of extracted substances with antioxidant properties and decreases the yield of ballast substances without complicating the procedure and increasing the consumption of materials.

Improved extraction of biologically active substances (BAS) from plant raw material is currently one of the most critical areas of scientific and technical development for producing extraction preparations [1]. Traditional extraction methods have reached their natural limit and cannot increase the rate of the process or the yield of final product. The effect of several force fields including ultrasound, electric, pulsed, electromagnetic [2], and others is used to improve the extraction of BAS from plant material.

Phenolic compounds are especially important BAS that are extracted from plants. Many experimental reports on the action of plant phenols indicate that they have a variety of effects on living organisms. However, wide use of plant phenols is limited by problems with their isolation from raw material.

We have shown previously [3] that maceration in a constant electric field may be successful for more complete extraction of phenolic antioxidants from medicinal plants. Passage of electric current through a mixture of water and bearberry leaves extracts substances from the plant raw material by the electrodialysis principle. Extracted substances are converted into ionized species through the action of current through a semi-permeable membrane, e.g., the plant cell wall. The yield of antioxidants increases at a certain current regime due to their increased internal diffusion from the plant cell. The temperature and thermal-treatment time of the extract can also be decreased. However, only some of the factors affecting the electroextraction, e.g., the electric field potential and extraction temperature and time, were studied in the previous work.

In continuation of the previous research, herein we study the effect of the extractant on extraction of phenolic antioxidants from bearberry leaves in an electric field and determine the optimum electroextraction method for providing the maximum yield of antioxidants from the plant material.

EXPERIMENTAL PART

Natural antioxidants (AO) were extracted from bearberry leaves by maceration in a constant electric field (potential U = 35 V, current I = 250 mA) at room temperature with constant stirring (Scheme 1). The extractants were aqueous acetic acid, HCl, and NaOH and various surfactants (SA). The resulting liquid extract was filtered, dried in a drying cabinet at 303 K for 3 d, and used in the dry form. The specific electrical conductivity (æ) of the extractants was determined by conductivity [4]. Extracts were standardized for amount of dry residue ($W_{D. R.}$, mass %) as determined by gravimetry [5]. The total amount of extracted phenolic compounds was characterized by the optical density (D) of the extract as determined by photocolorimetry at λ_{max} = 364 nm [6]. The antioxidant activity (AOA) of the extracts was studied using initiated oxidation [azodiisobutyronitrile (AIBN) initiator, T = 343 K] of commercial sunflower oil. The oil oxidation kinetics were followed using chemiluminescence [7].

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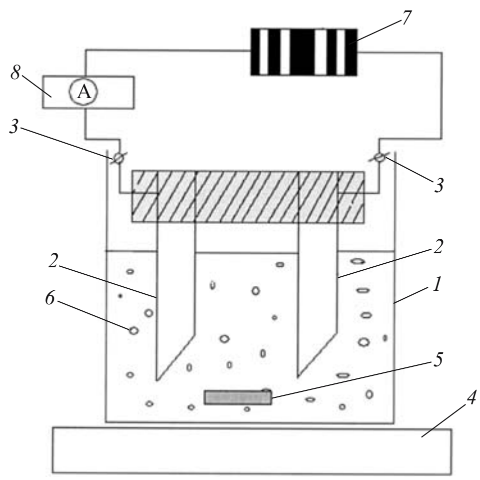
Scheme 1



Apparatus for extraction of natural antioxidants from plant raw material in a constant electric field: glass vessel (*1*), carbon electrodes (*2*), terminals for connecting to constant current source (*3*), stir bar (*4*), magnetic stirrer with heater (*5*), plant raw material (*6*), stabilized constant current source (*7*), ammeter (*8*).

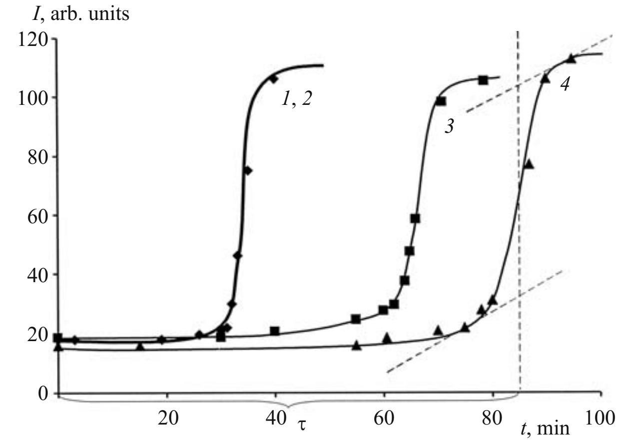


Fig. 1. Kinetic curves for the change of chemiluminescence intensity with initiated ([AIBN] = 2×10^{-2} M, [DBA] = 2×10^{-3} M, T = 343 K) oxidation of sunflower oil mixed with chlorobenzene (1:1) without additives (*1*) and with extracts of bearberry leaves (C = 0.1 mass %) produced in an electric field (U = 35 V, I = 250 mA) for the aqueous extract without an electric field (*2*), aqueous extract (*3*), and acetic-acid (1%) extract (*4*).

The chemiluminescence activator for initiated oxidation of sunflower oil was 9,10-dibromoanthracene (DBA). The AOA of the extracts was characterized by the quantity τ/τ_0, where τ is the induction period for oil oxidation in the presence of extract (0.1 mass %), and τ_0 is that without extract (Fig. 1).

RESULTS AND DISCUSSION

Electroextraction of phenols from plant raw material was improved by selecting extractants that could increase the yield of effective phenolic compounds and simultaneously accelerate the extraction. This was achieved by using solvents that increase the electrical conductivity of the extract and the solubility of the extracted substances and provide good wetting of the plant raw material.

We used aqueous solutions of acetic acid, HCl, and NaOH and SA. Aqueous solutions of acetic acid, HCl, and NaOH were used to extract BAS from medicinal plant raw material [6].

Table 1 gives data for the amount of extracted substances ($W_{D.\ R.}$, mass %), optical density (D), and AOA (τ/τ_0) of bearberry leaf extracts obtained by electroextraction using acetic-acid solutions of various concentration. It can be seen that the AOA, $W_{D.\ R.}$, and D of the extract increase with increasing acetic-acid concentration from 0.5 to 1%. The extract obtained using aqueous acetic acid (1%) had the greatest AOA. This is explained by the increased yield of active phenolic compounds from plant raw material with increasing electric conductivity of the solution (Fig. 2) and hydrolysis of extracted flavonoid glycosides to form the corresponding aglycons. Increasing the acid concentration further decreased the amount of extracted phenols and the AOA. This can be explained by the fact that increasing the concentration of acetic acid decreases its degree of dissociation and, therefore, the electrical conductivity (Fig. 2).

Using aqueous solutions of strong electrolytes, HCl and NaOH, as extractants did not produce extracts with higher AOA (Table 1). These extractants increase the total amount of extracted substances but do not apparently increase the yield of phenols and, therefore, do not increase the AOA of the extract. HCl increases extraction from the raw material of ballast substances that are not antioxidants. Furthermore, using NaOH solution increases extraction of polymeric phenols (tanning agents) that are weak antioxidants. Effective monomeric phenols are partially oxidized by alkaline extraction [6].

TABLE 1. Extractant Composition and Properties of Bearberry Leaf Extract Obtained by Maceration with Aqueous Acetic Acid, HCl, and NaOH in a Constant Electric Field (U = 35 V, I = 250 mA)

Aqueous solution	Concentration, %	$W_{D.\ R.}$, mass %	D	AOA, τ/τ_0
Acetic acid	0 (without electric field)	1.8	0.13	1.1
	0.5	3.73	0.20	1.6
	1	3.88	0.22	2.0
	1.5	4.10	0.21	1.9
	2	4.75	0.20	1.8
HCl	0.5	4.07	0.09	1.0
	1	4.25	0.12	1.0
NaOH	0.5	4.26	0.11	1.0
	1	4.75	0.14	1.0

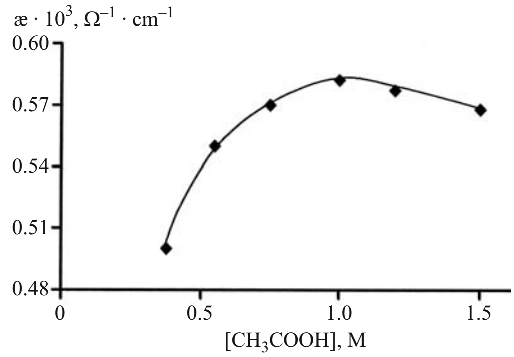


Fig. 2. Specific electrical conductivity (æ) of aqueous acetic-acid solutions as a function of CH_3CO_2H concentration.

Because solid-state extraction is related to penetration of extractant into the plant raw material and wetting of the plant cell contents, it seemed advisable to add to the extractant SA that decrease the surface tension at the interface of plant raw material and extractant and improve its wetting.

Table 2 shows the effect of various SA on the AOA of the acetic-acid extract of bearberry leaves. Cetylpyridinium chloride (CPC) was used as a cationic SA; sodium dodecylsulfate (SDS), as an anionic SA; Tween-80, as a nonionic SA.

It was found (Table 2) that adding to the extractant (1% aqueous acetic acid) SA, regardless of its nature, increased $W_{D.\,R.}$, D, and the AOA of the extract. Increasing the SA concentration in the extractant above a certain value that was different for each SA decreased the AOA of the resulting extracts. This may be due to an approach to the critical micelle-forming concentration (CMC). The most effective AO was the extract obtained using aqueous acetic acid (1%) with added Tween-80 (8.8×10^{-4} M), a nontoxic nonionic SA. Apparently this is explained by the fact that Tween-80 ($[H(CH_2CH_2O)_{20}O]_3[CH_2OOCC_{17}H_{33}]C_5H_6O$) contains a long hydrocarbon radical that is better than other SA for adsorbing on the surface of plant raw material.

Thus, the optimum extractant providing the highest extraction of natural AO from bearberry leaves by maceration in an electric field is aqueous acetic acid (1%) with added Tween-80 (8.8×10^{-4} M). The resulting extract (0.1 mass %) stabilizes sunflower oil to initiated oxidation by more than four times.

TABLE 2. $W_{D.\,R.}$, D, and AOA of Bearberry Leaf Extract as Functions of SA Concentration for Extraction in an Electric Field (U = 35 V, I = 250 mA). Solvent Aqueous Acetic Acid (1%)

SA	Concentration, M	$W_{D.\,R.}$, mass %	D	AOA, τ/τ_0
Sodium dodecylsulfate (SDS) $C_{12}H_{25}O_4SNa$	2.2×10^{-4}	3.84	0.19	3.0
	4.4×10^{-4}	3.82	0.19	3.5
	8.8×10^{-4}	3.85	0.25	4.3
	1.8×10^{-3}	3.32	0.24	3.8
	3.5×10^{-3}	3.08	0.20	2.6
Cetylpyridinium chloride (CPC) $C_{16}H_{33}NC_2H_5Cl$	1.5×10^{-5}	4.32	0.28	3.1
	2.9×10^{-5}	4.16	0.29	3.5
	5.8×10^{-5}	3.98	0.24	3.1
Polyhydroxyethylenesorbitan monooleate (Tween-80) $[H(CH_2CH_2O)_{20}O]_3[CH_2OOCC_{17}H_{33}]C_5H_6O$	4.4×10^{-4}	4.32	0.24	2.3
	8.8×10^{-4}	3.75	0.30	4.7
	1.8×10^{-3}	3.65	0.31	3.2
	3.5×10^{-3}	3.22	0.30	1.8

REFERENCES

1. A. B. Golovanchikov and M. V. Popov, *Khim.-farm. Zh.*, **32**, No. 8, 31 – 33 (1998).
2. V. D. Ponomarev, *Extraction of Medicinal Raw Material* [in Russian], Meditsina, Moscow (1976), pp. 123 – 137.
3. N. I. Belaya, T. A. Filippenko, A. V. Belyi, et al., *Khim.-farm. Zh.*, **40**, No. 9, 42 – 44 (2006).
4. Ya. I. Gerasimov, ed., *Course in Physical Chemistry* [in Russian], Khimiya, Moscow (1973), pp. 397 – 399.
5. N. I. Bazykina, A. N. Nikolaevskii, T. A. Filippenko, and V. G. Kaloerova, *Khim.-farm. Zh.*, **36**, No. 2, 46 – 49 (2002).
6. A. Blazhei and L. Shutyi, *Phenolic Compounds of Plant Origin* [in Russian], Nauka, Moscow (1977), pp. 110 – 239.
7. V. Ya. Shlyapintokh, O. N. Karpukhin, L. M. Postnikov, et al., *Chemiluminescent Methods for Investigating Slow Chemical Processes* [in Russian], Nauka, Moscow (1966), pp. 91 – 130.