PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2021, **55**(1), p. 25–38

Biology

ANTIOXIDANT POTENTIAL OF SOME HERBS REPRESENTED IN ARMENIAN FLORA AND CHARACTERIZATION OF PHYTOCHEMICALS

M. M. GINOVYAN [*], N. Zh. SAHAKYAN [**], M. T. PETROSYAN [***], A. H. TRCHOUNIAN

Chair of of Biochemistry, Microbiology and Biotechnology, YSU, Armenia

Adverse effect of oxidative stress is a huge problem in medicine. In several circumstances, exogenous antioxidants are needed to regulate the amount of reactive species in the body. Plants are considered as a promising source for new antioxidant compounds. The goal of this study was to evaluate the antioxidant potential of extracts of the following herbs: *Agrimonia eupatoria*, *Hypericum alpestre*, *Rumex obtusifolius* and *Sanguisorba officinalis* using different chemical-based tests. GC-MS technique was used for identification of plant's volatile bio-active constituents. The results revealed good potential of the tested herbs as sources for new antioxidant compounds.

https://doi.org/10.46991/PYSU:B/2021.55.1.025

***Keywords*:** antioxidant potential, plant extract, Armenian flora, total phenolic content, *Rumex obtusifolius*, GC-MS analysis.

Introduction. Oxidative stress is a misbalance between the free reactive oxygen/nitrogen species (ROS/RNS) and ability of organism's antioxidant defense systems to counteract them [1]. Adverse effect of oxidative stress is a huge problem and of great importance. Oxidative stress, induced by free radicals, is responsible for a number of chronic and degenerative diseases in human. Particularly, involvement of oxidative stress in development of more than 100 diseases, including cancer, cardiovascular, neurodegenerative (Parkinson, Alzheimer, Huntington, etc.), inflammatory diseases, aging, *etc*. has been reported [2, 3].

Although different effective synthetic antioxidant compounds have been developed, their use is restricted in a number of countries due to various side effects. Hence, currently more attention has given to the discovery of antioxidant compounds of natural origin which can be used for regulation of oxidative stress, as well as in the food processing [4, 5]. Plants are considered as one the most important source for exogenous antioxidant compounds [4, 6]. It is deemed that plant compounds possessing antioxidant properties include: various vitamins, carotenoids, a variety of phenolic compounds (stilbens, phenolic acids, tannins, flavonoids, anthocyanins), fatty acids, alkaloids, amines, lutein, ubiquinone, *N*-acetylcysteine, etc. [5, 7].

[*] E-mail: mikayel.ginovyan@ysu.am
[**] E-mail: sahakyannaira@ysu.am
[***] E-mail: margaritpetrosyan@ysu.am

The Armenian flora is rich in herb species, which have been widely used in traditional medicine since ancient times [8]. However, this biodiversity has not been studied properly for their biological activity, including antioxidant properties. Therefore, there could be hidden a great potential for their antioxidant properties, which can be of huge importance for therapeutic use as well as for the food industry. In the previous research works of our group, various parts of 28 wild herbs of Armenian flora were screened for their antimicrobial properties. Based on data obtained, the following plant species were selected taking into account their promising antimicrobial properties: *Agrimonia eupatoria*, *Hypericum alpestre*, *Rumex obtusifolius* and *Sanguisorba officinalis* [9]. High antibacterial, antifungal, antiviral and antibiotic modulatory activity of different extracts of *A. eupatoria, H. alpestre, S. officinalis, R. obtusifolius* has also been shown by our group [8–12]. All selected plant species are well-known herb species widely used in traditional medicine. In particular, they have been used for treatment of various medical conditions, which may indicate their antioxidant action according to Armenian Herbal Medicine Directory books [13, 14]. Taking into account promising biological activities of these four plant materials, it was also very interesting to evaluate their antioxidant potential.

The aim of this research was to evaluate antioxidant potential of different extracts *of A. eupatoria, H. alpestre, S. officinalis and R. obtusifolius* as a new source for antioxidant compounds and reveal the correlation of their antioxidant capacity with the chemical composition.

Materials and Methods.

Collection of Plant Material. Four herbs from Armenian flora were investigated based on initial screening for biological activity [9]. All plant species were collected from Tavush region (1300–1600 *m* above sea level). The collection, identification and preparation of plant material were done according to the already established protocol [8]. The plants were deposited to the Herbarium of Yerevan State University. The following plant materials were used: *A. eupatoria* L. (whole plant) (voucher specimen number ERCB 13207), *H. alpestre* subsp. *polygonifolium* (Rupr.) Avet. & Takht. (aerial part) (ERCB 13206), *R. obtusifolius* L. (seed) (ERCB 13208), *S. officinalis* L. (aerial part) (ERCB 13205).

Preparation of Plant Crude Extracts. Plant crude extracts were prepared by maceration technique using methanol (98%) and acetone (99.8%), according to method described in [8]. Dry crude extracts were stored in freezer (–18 to –20°C).

Evaluation of Antiradical Activity by DPPH Assay. The antiradical activity of the tested plant crude extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [15]. The test solutions contained 250 μL (1 mM) DPPH, 750 μL ethanol (96%) and 1000 μL plant crude extract at various concentrations. Catechin was used as a positive control. The absorbance was measured at 517 *nm* wavelength after 30 *min* incubation of mixture at 25°C. Radical scavenging activity (%) was measured using the following formula: Antiradical activity (%)= $\frac{A_c - A_t}{A_c} \times 100$, where A_c is the absorbance of the control (DPPH without adding the test solution), A_t is the sample absorbance. IC_{50} values were also determined for each plant extract. The thermal stability of plants crude extracts' antioxidant compounds was tested by DPPH assay. The dried plant extracts were kept at temperatures of 60°C, 80°C,

100°C and 121°C for 30 *min*. Then the samples were cooled at room temperature and stored in a freezer at –18°C to –20°C. Afterwards, the residual antiradical activity of the plant extracts was tested.

Hydrogen Peroxide Reducing Activity. The hydrogen peroxide (H_2O_2) reducing ability of plant extracts was evaluated according to the method described by Ruch et al. [16]. 40 *mM* H_2O_2 solution was prepared in 50 *mM* phosphate buffer (pH 7.4). H_2O_2 concentration was measured by a spectrophotometer at 230 *nm* wavelength. 0.2 *mL* of plant extract with a concentration of 1000 $\mu g\ mL^{-1}$ was added to 1.8 *mL* H_2O_2 and left for 10 *min*. Then absorption was measured compared with the phosphate buffer as a blank. The percent reduction of hydrogen peroxide was calculated by the following formula: H_2O_2 reduction$= \frac{A_c - A_t}{A_c} \times 100\%$, where A_c is the absorption of the control solution, A_t is the absorption of the test solution. Ascorbic acid was used as a positive control at a concentration of 10 $\mu g\ mL^{-1}$.

Metal Chelating Activity. Ferrozine can form a red color complex with Fe^{2+} ions. This interaction is reduced with the presence of other chelating agents. The metal chelating activity can be assessed by color change due to the formation of ferrozine–Fe complexes [17]. 0.4 *mL* of plant extract was added to 1 *mL* of ferrous chloride (0.2 *mM*) and left for 10 *min*. The interaction began after adding 0.4 *mL* of ferrozine (5 *mM*). After 10 *min* of incubation at room temperature, the absorption was measured at a wavelength of 562 *nm*. Percent metal chelating activity of plant extracts was calculated according the following formula: Chelating activity$= \frac{A_c - A_t}{A_c} \times 100\%$, where A_c is the absorption of the control solution, A_t is the absorption of the test solution. Ethylenediaminetetraacetic acid (EDTA) at 22 $\mu g\ mL^{-1}$ concentration was used as a positive control.

Determination of Extent of Lipid Peroxidation (MDA). The effect of plant extracts on extent of lipid peroxidation of rat brain homogenate evaluated by TBARS assay [18]. Mice brain tissue homogenate (in 20 *mM* phosphate buffer (pH 7.4)) used during the test. Test solution contains 0.3 *mL* of plant extract (3 $mg\ mL^{-1}$ concentration), 0.7 *mL* 250 *mM* HCl, 1 *mL* tissue homogenate and 2 *mL* TBA solution (0.375%). The absorbance of mixtures measured at 532 *nm*. Malondialdehyde concentration calculated using an extinction coefficient of $1.56\times10^5\ M^{-1}\ cm^{-1}$.

Identification of the Chemical Composition of Plant Crude Extracts Using GC-MS Technique. For the identification of volatile compounds contained in tested plant crude extracts gas chromatography (GC) technique combined with mass selective (MS) analysis was applied using a Hewlett–Packard 5890 Series II gas chromatograph, fitted with a fused silica HP – 5MS capillary column (30 *m* × 0.25 *mm*, with thickness of 0.25 μm) [19]. The oven temperature was varied from 40 to 250°C with the scanning rate of 3°C/*min*. Helium (purity 5.6) was used as a carrier gas at a flow rate of 1 *mL*/*min*. The GC was equipped with a Hewlett–Packard 5972 Series MS detector. The MS operating parameters were an ionization voltage of 70 *eV* and an ion source temperature of 250°C. The diluted samples of the extracts (1/100, v/v in HPLC methanol) with a volume of 1 μL were injected manually. The identification of peaks was tentatively carried out based on library search using National Institute of Standards and Technology (NIST)-2013.

Data Processing. All experiments were independently repeated three times. The data obtained were processed; mean values and standard deviations were calculated using GraphPad Prism 8.0.1 (GraphPad Software, Inc.; USA) software. The results obtained in the study are reliable ($p<0.05$), unless another value is followed. Pearson's correlation test was used to determine the correlation between total phenolic content (TPC) and antioxidant activities obtained by different assays.

Results.

To assess the antioxidant potential of the tested plant extracts, various chemical-based methods were used, which made it possible to reveal the potential of *A. eupatoria, H. alpestre, S. officinalis* and *R. obtusifolius* as a source of new antioxidant compounds.

***DPPH Radical Scavenging Activity*.** According to data obtained, methanol and acetone extracts of the tested plant materials possessed high antiradical activity (Tab. 1). DPPH scavenging IC_{50} values of plant methanol extracts were determined. It was revealed that the methanol extract of *R. obtusifolius* had lowest IC_{50} value (25.29 $\mu g\ mL^{-1}$ at a concentration of 0.05 $mg\ mL^{-1}$). This was nearly double the IC_{50} values of other herbal extracts tested. The effect of heat treatment on the antiradical activity of plant extracts was also tested. The data obtained revealed that antioxidant compounds of the tested plant methanol and acetone extracts completely retained their DPPH scavenging activity even after heat treatment at 121°C for 30 *min*.

Table 1

Antioxidant activity of the tested plant methanol and acetone extracts using different chemical-based methods

Plant name	Extract	DPPH reduction		H_2O_2 %-reduction in the presence of 100 $\mu g\ mL^{-1}$ plant crude extract	Fe^{2+} %-chelation in the presence of 100 $\mu g\ mL^{-1}$ plant crude extract	MDA %-reduction in the presence of 225 $\mu g\ mL^{-1}$ plant crude extract	Total phenolic compounds, μg GAE (mg DW)$^{-1}$
		%-reduction at 100 $\mu g\ mL^{-1}$ plant crude extract	IC_{50} value, $\mu g\ mL^{-1}$				
A. eupatoria	methanol	94.53±1.3	40.74±1	42.07±1.4	37.30±1.2	–	358.9±0.62
	acetone	98.15±1.2	ND	5.79±0.8	8.73±0.9	–	348.34±0.97
H. alpestre	methanol	81.75±1.1	50.8±1.6	99.9 ±1.0	31.75±1.4	4.48±0.5	263.3±0.61
	acetone	98.52±1.3	ND	98.30±1.1	–	2.98±0.7	209.87±0.63
R. obtusifolius	methanol	91.97±0.9	25.29±0.8	40.78±0.9	73.02±1.6	–	327.2±0.33
	acetone	88.89±.1.0	ND	99.30±2.1	5.56±1.0	–	273.83±0.28
S. officinalis	methanol	86.86±2.1	54.94±1.2	–	41.27±1.6	19.40±0.9	92.6±0.4
	acetone	96.30±1.4	ND	9.0±0.8	–	29.85±1.1	228.99±0.33
Positive control		NA	3 (Catechin)	11.23±0.9 (Ascorbic acid)	28.57±2.1 (EDTA)	91.1±1.4 (Tocopherol)	NA

"–" absence of activity; ND – not determined; NA – not applicable. All experiments were independently repeated in triplicate. The data are presented as the mean ± SD, $p<0.05$.

Hydrogen Peroxide Reducing Activity. The data obtained showed that some of the tested plant methanol and acetone extracts possessed high reducing activity for hydrogen peroxide (Tab. 1). At a concentration of 100 $\mu g\ mL^{-1}$, the highest hydrogen peroxide reducing activity was observed in *H. alpestre* methanol and acetone extracts (99.9% and 98.30%, respectively) and *R. obtusifolius* acetone extracts (99.30%) (the concentration of H_2O_2 in the mixture was 36 *mM*). Methanol extracts of *A. eupatoria* and *R. obtusifolius* exhibited moderate activity leading to the hydrogen peroxide reduction by 42.07% and 40.78%, respectively. Acetone extracts of *A. eupatoria* and *S. officinalis* possessed relatively low activity for the reduction of H_2O_2. At the concentrations tested, methanol extract of *S. officinalis* exhibited no activity in reducing hydrogen peroxide.

Metal Chelating Activity. According to the data obtained, it was revealed that some of the tested plant extracts had considerable metal chelating activity (Tab. 1). Particularly, *A. eupatoria*, *H. alpestre*, *R. obtusifolius* and *S. officinalis* methanol extracts exhibited an expressed metal chelating activity at a concentration of 125 $\mu g\ mL^{-1}$ by reducing the number of Fe^{2+}–ferrozine complexes by 37.30%, 31.75%, 73.02%, 41.27%, respectively (in the presence of 0.125 *mM* $FeCl_2$). Acetone extracts of the tested plant materials had low metal chelating ability at tested concentrations. Acetone extracts of *H. alpestre* and *S. officinalis* have not exhibited metal chelating ability.

TBARs Assay. Anti-peroxidative activity of tested plant extracts was evaluated by determining their ability to reduce MDA formation level. Only two of tested plant materials have brought to reduction of MDA formation at 225 $\mu g\ mL^{-1}$ concentration (Tab. 1). Moreover, *H. alpestre* methanol and acetone extracts caused only slight reduction of MDA level. Methanol and acetone extracts of *S. officinalis* exhibited anti-peroxidative activity by reducing MDA level by 19.40% and 29.85% respectively.

Table 2

Compounds identified in the crude methanol extract of A. eupatoria

Identified compound	Retention time	% Peak area	Compound nature	Biological activities
Isosteviol	4.106	5.54	tetracyclic diterpenoid	antibacterial, antifungal, antibiofilm, anti-inflammatory, antioxidant, anticancer
1,19-Eicosadiene	37.893	3.9	alkene	not reported
Palmitic acid	41.356	27.44	fatty acid	antibacterial, antioxidant, pesticidal, antinematodal
1-Methoxy-3-(2-hydroxyethyl)nonane	44.467	3.84	carbohydrate	not reported
7,10,13-Hexadecatrienoic acid, methyl ester	45.212	21.43	fatty acid methyl esters	not reported
E-9-Tetradecenal	45.475	0.44	fatty aldehydes	not reported
Octadecanoic acid	45.847	5.16	fatty acid	antimicrobial
Eicosane	46723	0.72	alkane	not reported
Nonadecane	48.892	0.62	alkane	not reported
1-chloroeicosane	52.934	0.55	–	not reported
1-Octadecene	54.237	0.40	alkene	not reported
Squalene	56.438	4.52	triterpene	antibacterial, antioxidant, antitumor, pesticidal
None pure ingredients		10.65		

GC-MS Analysis of the Tested Plants Methanol Extracts. In the methanol extracts of selected tested plant materials, various biologically active compounds were identified by GS-MS analysis, which could play important role in their antioxidant effect. According to the data obtained, 13 compounds were identified in the methanol extract of *A. eupatoria*. The identified compounds, their exact molecular mass, retention time, quantity, nature, and biological activities are presented in Tab. 2.

Table 3

Compounds identified in the crude methanol extract of H. alpestre

Identified compound	Retention time	% Peak area	Compound nature	Biological activities
Furfural	4.411	1.44	furan aldehyde	antibacterial
Guaiacol	9.888	0.56	phenolics	antimicrobial, antioxidant
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	11.564	8.62	–	antioxidant, antimicrobial, anti-inflammatory
Catechol	12.418	2.9	phenolics	antifungal, antibacterial, antioxidant, antiviral, antitumor
4-Hydroxy-3-methylacetophenone	13.163	0.60	phenolics	not reported
Dodecanoic acid	16.394	1.22	fatty acid	antimicrobial
Vanillic acid	17.117	3.70	phenolics	antioxidant, antibacterial
Tetradecanoic acid	19.099	7.69	fatty acid	antimicrobial
Pentadecanoic acid	20.698	0.49	fatty acid	not reported
Hexadecanoic acid, methyl ester	21.673	0.20	fatty acid methyl esters	antioxidant, pesticidal, antinematodal
Palmitic acid	22.834	9.46	fatty acid	antibacterial, antioxidant, pesticidal, antinematodal
cis,cis,cis-7,10,13-Hexadecatrienal	23.163	0.73	fatty aldehydes	not reported
2-Methyl-Z,Z-3,13-octadecadienol	24.367	0.26	–	not reported
Phytol	25.298	0.52	diterpen	antimicrobial, anticancer, antioxidant, anti-inflammatory
Linolenic acid	26.087	17.44	fatty acid	antibacterial
Octadecanoic acid	26.591	2.15	fatty acid	antimicrobial
Eicosane	27.226	0.13	alkane	not reported
Tritetracontane	29.198	0.68	alkane	insecticide
N-[4-bromo-n-butyl]-2-Piperidinone	30.260	0.35	alkaloids	antimicrobial, antioxidant, anti-inflammatory
Tetradecane	32.341	0.29	alkane	not reported
Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-	33.776	0.27	alkaloids	not reported
Curan-17-oic acid, 2,16-didehydro-20-hydroxy-19-oxo-, methyl ester	35.068	6.84	–	not reported
Hexadecane	35.244	1.12	alkane	not reported
trans-Farnesol	36.427	0.48	sesquiterpenoids	antimicrobial, antibiotic modulating activity, anti-biofilm
None pure ingredients		1.95		

Based on literature data, it can be assumed that palmitic acid, squalen and isosteviol can contribute to the antioxidant activity of crude methanol extract of *A. Eupatoria* [20]. 24 compounds were identified in the crude extract of *H. alpestre* (Tab. 3).

The following compounds can highly contribute to the antioxidant activity of *H. alpestre* extracts: catechol, guaiacol, Vanillic acid and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one, which possess antioxidant properties according to literature data [21–26]. In the crude extract of *R. obtusifolius*, 21 compounds were identified. The identified compounds and their characteristics are shown in Tab. 4.

Table 4

Compounds identified in the crude extract of R. obtusifolius

Identified compound	Retention time	% Peak area	Compound nature	Biological activities
1,2,4-Benzenetriol	15.540	0.46	phenolics	antiseptic, fungicidal, insecticide, antioxidant
Tritetracontane	19.703	0.12	alkane	insecticide
Hexadecanoic acid, methyl ester	21.619	0.30	fatty acid methyl esters	antioxidant, pesticidal, antinematodal
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester (Butyl isobutyl phthalate)	21.893	0.54	–	not reported
Palmitic acid	22.572	12.25	fatty acid	antibacterial, antioxidant, pesticidal, antinematodal
Hexadecanoic acid, ethyl ester	22.879	1.29	fatty acid ethyl esters	antioxidant, anti-nematodal, pesticidal, hemolytic
Eicosane	23.251	0.19	alkane	not reported
Oleic Acid	24.018	3.65	fatty acid	antimicrobial
Methyl linoleate	24.686	0.45	fatty acid	antifungal, antitumor
11-Octadecenoic acid, methyl ester	24.850	0.68	fatty acid methyl esters	not reported
3,8-Dimethyldecane	25.168	0.16	alkane	not reported
Linoleic acid	26.022	41.32	fatty acid	antibacterial
cis-Vaccenic acid	26.209	24.43	fatty acid	antimicrobial
4-Methyldocosane	29.11	0.15	alkane	not reported
N-[4-bromo-n-butyl]-2-Piperidinone	31.050	0.64	alkaloids	antimicrobial, antioxidant, anti-inflammatory
1-Heptadecene	32.923	0.32	alkenes	not reported
cis-9-Hexadecenoic acid	33.668	0.40	fatty acid	antibacterial
Octadecane	34.204	0.27	alkane	not reported
9-Octadecenal, (Z)-	34.872	0.53	fatty aldehydes	antimicrobial
Heptacosane, 1-chloro-	35.190	0.31		not reported
Supraene	36.351	0.21	triterpenoids	antibacterial, antioxidant, pesticidal, antitumor
None pure ingredients		2.58		

Palmitic acid and hexadecanoic acid, ethyl ester may contribute to the antioxidant activity of this plant methanol extracts. In the crude methanol extract of *S. officinalis*, 18 compounds were identified (Tab. 5). Palmitic acid may contribute greatly to the antioxidant activity of crude methanol extract of *S. officinalis*. Antioxidant activity of palmitic acid were reported in many research works [27, 28].

Table 5

Compounds identified in the crude methanol extract of S. officinalis

Identified compound	Retention time	% Peak area	Compound nature	Biological activities
Furfural	4.017	5.87	furan aldehyde	antimicrobial, pesticidal, fungicidal, antinematodal, insecticidal
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	11.421	3.71	–	not reported
5-(Hydroxymethyl)furfural	13.042	28.83	furan compound	antimicrobial
Dodecanoic acid	16.262	1.08	fatty acid	antimicrobial
β-D-Glucopyranoside, methyl	19.680	13.11	sugar	not reported
n-Capric acid isopropyl ester	20.556	0.84	fatty acid esters	not reported
Palmitic acid	22.604	4.34	fatty acid	antibacterial, antioxidant, pesticidal, antinematodal
2-Methyl-Z,Z-3,13-octadecadienol	23.546	0.09	–	not reported
Methyl linolenate	24.718	0.08	fatty acid	antifungal, antitumor
Linolenic acid	25.857	5.33	fatty acid	antibacterial
cis-Vaccenic acid	26.054	0.20	fatty acid	antimicrobial
Octadecanoic acid	26.317	0.78	fatty acid	antimicrobial
Tricosane	29.077	0.09	alkane	not reported
2-Piperidinone, N-[4-bromo-n-butyl]-	31.027	0.13	alkaloids	antimicrobial, antioxidant, anti-inflammatory
Tetradecane	32.232	0.15	alkane	not reported
Nonadecane	32.911	0.08	alkane	not reported
10-Methylnonadecane	35.178	0.08	alkane	not reported
Squalene	36.328	0.12	triterpene	antibacterial, antioxidant, antitumor, pesticidal
None pure ingredients		1.11		

Discussion. The study attempted to evaluate four herbs from Armenian flora for their antioxidant properties. For this purpose, various *in vitro* chemical-based methods were used. According to the DPPH assay, all tested plant extracts possessed high antiradical activity. Some literature data confirmed our results. Kubínová with co-workers [29] reported that *A. eupatoria* had the highest anti-radical activity (methanol extract of *A. eupatoria* at a concentration of 2 $mg\ mL^{-1}$ led to 63.09% reduction of DPPH) within the 5 species of the genus *Agrimonia*. However, these results are significantly lower than the data obtained in our experiments. In another study [30], acetone extract of *A. eupatoria* at a concentration of 125 $\mu g\ mL^{-1}$ resulted in 94.83% inhibition of DPPH, which is only slightly different from obtained data (98.15% reduction at a concentration 100 $\mu g\ mL^{-1}$). High antiradical activity of the

extracts of *H. alpestre* was shown for the first time. However, the high anti-radical activity of other species of the genus is well-known [31]. Harshaw et al. [32] showed that in various extracts of *R. obtusifolius* (methanol, hexane, dichloromethane), the methanol extract had the highest DPPH scavenging activity, with IC_{50} value of slightly inferior to obtained values. In another study [33], it was reported that leaf and root extracts of *R. dentatus* possessed high anti-radical activity (the highest values were obtained in the cases of root ethyl acetate and root ethanol extracts, IC_{50} values of which were 12 $\mu g\ mL^{-1}$ and 152 $\mu g\ mL^{-1}$, respectively). According to the literature data [34], IC_{50} value of *S. officinalis* methanol extract was 2.95 $mg\ mL^{-1}$ (the DPPH concentration in the reaction mixture was 0.5 $mg\ mL^{-1}$), which significantly exceeds the value 54 $\mu g\ mL^{-1}$ we obtained (DPPH concentration in the reaction mixture was 0.05 $mg\ mL^{-1}$). Another study [35] reported that the IC_{50} value of *S. officinalis* methanol extract was 4.1 $\mu g\ mL^{-1}$ (DPPH concentration was 0.01 $mg\ mL^{-1}$), which was lower than the value we obtained. Thus, we can conclude that the investigated plant methanol and acetone extracts possessed high DPPH scavenging activity; besides, IC_{50} value of *R. obtusifolius* was almost two times higher than of other tested plant extracts.

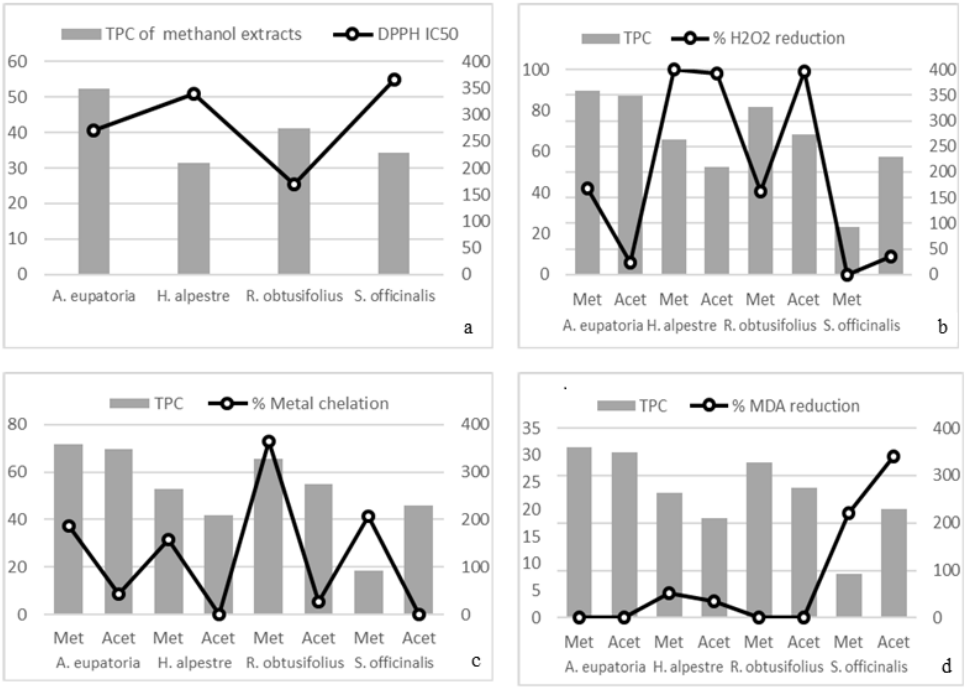
Hydrogen peroxide itself is not highly reactivity, but it can be toxic to cells as it generates highly reactive hydroxyl radicals in cells [36]. Therefore, the neutralization of H_2O_2 can have an important effect on oxidative stress-protective functions. The data obtained showed that three of the tested plants, with the exception of *S. officinalis*, have high reducing activity for hydrogen peroxide, and this activity differs depending on the solvent. Moreover, H_2O_2 reducing activity of *H. alpestre, R. obtusifolius* and *S. officinalis* was investigated for the first time. The literature contains data on the H_2O_2 reducing activity of some representatives of the genus *Rumex* (chloroform, ethyl acetate and methanol extracts of *R. hastatus* D. [36]. In another study, the relatively low H_2O_2 reducing activity of the *A. eupatoria* water-ethanol extract was shown, which partly coincides with the obtained data [37]. H_2O_2 reducing activity of various species of the genus *Hypericum* has been reported. In particular, H_2O_2 reducing activity of ethanol and water extracts of *H. venustum* was shown in [38].

The ability to chelate metal ions also plays an important role in the mechanisms of antioxidant activity, since they can catalyze the decomposition of hydrogen peroxide, as well as Fenton-type reactions [36]. In this study, considerable metal chelating activity of some of the tested plant extracts was shown. In addition, metal chelating activity of *H. alpestre, Sanguisorba* spp. and *Rumex* spp. was shown for the first time. In the literature, there are some data about metal chelating properties of some representatives of the tested plant genera. In particular, Kizil et al. [39] demonstrated low metal chelating activity of ethanol extracts of *H. triquetrifolium* and *H. scabroides*, which partly coincides with the data obtained, as *H. alpestre* acetone extract did not exhibit metal chelating activity. In another study [38], it was reported that ethanol and water extracts of *H. venustu* possessed metal chelating activity. Metal chelating ability of *A. eupatoria* extracts was also reported [37]. Hence, methanol extracts of the tested plant materials can be a potential source of metal chelators. It is important to point out that *R. obtusifolius* methanol extract was almost twice as active as the other three plants.

According to the data obtained, within the tested plant materials, only *S. officinalis* extracts possessed considerable anti-peroxidative activity. In the literature, there is no data on anti-peroxidative activity of the tested plant extracts, determined by TBARS assay.

For the possible use of plant biologically active extracts and compounds, it is also important to determine thermostability of the compounds responsible for their antioxidant activity. The obtained data revealed that the compounds responsible for antiradical activity of all tested plant extracts were thermostable.

We tried to find a correlation between the total phenolic content (TPC) in the tested plant materials, which were determined previously [12], and their antioxidant activity. A considerable negative correlation was found between the TPC of extracts and DPPH scavenging IC_{50} values of the ested plant extracts (Peterson's coefficient, $R = –0.49$) (see Fig., a). This means that the higher the TPC, the higher DPPH scavenging activity. A linear correlation between DPPH scavenging activity and TPC was shown in many research works [40, 41]. However, the correlation depends on plants samples, as other researchers have not found a significant correlation between TPC and DPPH scavenging activity [40, 41]. No correlations were obtained between TPC and H_2O_2 reduction activity ($R = 0.049$) and metal chelating activity (R=0.13). Moreover, a negative correlation was found between TPC and MDA reduction activity ($R = –0.9$) (see Fig., b–d). Therefore, it can be speculated that non-phenolic compounds of the tested plant materials can make a significant contribution to their H_2O_2 reduction, metal chelating and MDA reduction activities.



Correlation of total phenolic content (TPC) and DPPH sub-inhibitory concentrations (a), hydrogen peroxide percentage reduction (b), metal chelating percentage reduction (c) and MDA percentage reduction activity (d) of tested plant methanol and acetone extracts (for details, see Materials and Methods).

GS-MS analysis of crude methanol extracts of the tested plant materials allowed the identification of many compounds which can have contribution on their antioxidant activity.

Conclusion. Thus, based on different *in vitro* antioxidant assays, it was demonstrated that *A. eupatoria, H. alpestre, R. obtusifolius and S. officinalis*, which are herbs widely used in food and traditional medicine, have good potential as a source for new antioxidant compounds. *R. obtusifolius* can be used as a nutritious food for its exogenous antioxidants. Moreover, it was shown that antioxidant compounds contained in tested plant extracts were thermostable, which is important in prospect of their further possible use. Particularly, high antioxidant activity of extracts of *R. obtusifolius* was demonstrated. Antioxidant activity of *H. alpestre* reported for the first time. Considerable correlation was found between DPPH scavenging activity of the tested plant extracts and the total phenolic contents. It was hypothesized that non-phenolic compounds may have a considerable contribution to H_2O_2 reduction, metal chelating and MDA reduction activities of the tested plant extracts. GC-MS analysis made it possible to identify active antioxidant compounds in methanol extracts of the tested plant materials.

The authors would like to thank Dr. Samvel Aloyan from Nairian CJSC (Armenia) for his help in conducting GC-MS analysis, which was implemented in the framework of scientific cooperation with the natural cosmetics producing company “Nairian” (Armenia).

This work was supported by the Science Committee of the MESCS RA, in the frames of Basic support to Research Institute of Biology, Yerevan State University, and Research grants Nos. 18T-1F267 and 19YR-1F042.

Received 16.02.2021
Reviewed 14.04.2021
Accepted 27.04.2021

REFERENCES

1. Persson T., Popescu B.O., Cedazo-Minguez A. Oxidative Stress in Alzheimer’s Disease: Why Did Antioxidant Therapy Fail? *Oxid. Med. Cell. Longev*. **2014** (2014), 1–11. https://doi.org/10.1155/2014/427318
2. Khatoon M., Islam E., et al. Estimation of Total Phenol and *in vitro* Antioxidant Activity of *Albizia procera* Leaves. *BMC Res. Notes* **6** (2013), 121. https://doi.org/10.1186/1756-0500-6-121
3. Gutteridge J.M.C., Halliwell B. Mini-Review: Oxidative Stress, Redox Stress or Redox Success? *Biochem. Biophys. Res. Commun*. **502** (2018), 183–186. https://doi.org/10.1016/j.bbrc.2018.05.045
4. Rasooli I. Food Preservation – A Biopreservative Approach. *Food* **1** (2007), 111–136.
5. Nićiforović N., Mihailović V., et al. Antioxidant Activity of Selected Plant Species; Potential New Sources of Natural Antioxidants. *Food Chem. Toxicol*. **48** (2010), 3125–3130. https://doi.org/10.1016/j.fct.2010.08.007

6. Amorati R., Valgimigli L. Methods to Measure the Antioxidant Activity of Phytochemicals and Plant Extracts. *J. Agric. Food Chem.* **66** (2018), 3324–3329. https://doi.org/10.1021/acs.jafc.8b01079
7. Pisoschi A.M., Pop A. The Role of Antioxidants in the Chemistry of Oxidative Stress: A Review. *Eur. J. Med. Chem*. **97** (2015), 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040
8. Ginovyan M., Petrosyan M., Trchounian A. Antimicrobial Activity of Some Plant Materials Used in Armenian Traditional Medicine. *BMC Complement. Altern. Med*. **17** (2017), 50. https://doi.org/10.1186/s12906-017-1573-y
9. Ginovyan M., Trchounian A. Screening of Some Plant Materials Used in Armenian Traditional Medicine for Their Antimicrobial Activity. Proc. YSU B: *Chem. Biol. Sci*. **51** (2017), 44–53. https://doi.org/10.46991/PYSU:B/2017.51.1.044
10. Ginovyan M. Effect of Heat Treatment on Antimicrobial Activity of Crude Extracts of Some Armenian Herbs. *Proc. YSU B: Chem. Biol. Sci.* **51** (2017), 113–117. https://doi.org/10.46991/PYSU:B/2017.51.2.113
11. Ginovyan M., Trchounian A. Novel Approach to Combat Antibiotic Resistance : Evaluation of Some Armenian Herb Crude Extracts for Their Antibiotic Modulatory and Antiviral Properties. *J. Appl. Microbiol*. **127** (2019), 472–480. https://doi.org/10.1111/jam.14335
12. Ginovyan M., Ayvazyan A., et al. Phytochemical Screening and Detection of Antibacterial Components from Crude Extracts of Some Armenian Herbs Using TLC-Bioautographic Technique. *Curr. Microbiol*. (2020), 1223–1232. https://doi.org/10.1007/s00284-020-01929-0
13. Torosyan A. *Armenian Herbs*. Yerevan, Hayastan (1983).
14. Harutyunyan H. *Herbs from Armenian Medieval Medical Guides*. Yerevan, Luys (1990).
15. Apak R., Gorinstein S., et al. Methods of Measurement and Evaluation of Natural Antioxidant Capacity/Activity (IUPAC Technical Report). *Pure Appl. Chem.* **85** (2013), 957–998. https://doi.org/10.1351/PAC-REP-12-07-15
16. Ruch R.J., Cheng S.J., Klaunig J.E. Prevention of Cytotoxicity and Inhibition of Intercellular Communication by Antioxidant Catechins Isolated From Chinese Green Tea. *Carcinogenesis* **10** (1989), 1003–1008. https://doi.org/10.1093/carcin/10.6.1003
17. Alam Md.N., Bristi N.J., Rafiquzzaman M. Review on *in vivo* and *in vitro* Methods Evaluation of Antioxidant Activity. *Saudi Pharm. J.* **21** (2013), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002
18. Kulisic T., Radonic A., et al. Use of Different Methods for Testing Antioxidative Activity of Oregano Essential Oil. *Food Chem*. **85** (2004), 633–640. https://doi.org/10.1016/j.foodchem.2003.07.024
19. Avetisyan A., Markosian A., et al. Chemical Composition and Some Biological Activities of the Essential Oils from Basil *Ocimum* Different Cultivars. *BMC Complement. Altern. Med*. **17** (2017), 60. https://doi.org/10.1186/s12906-017-1587-5
20. Wu Y., Dai G.F., et al. Stereoselective Synthesis of 15- and 16-Substituted Isosteviol Derivatives and Their Cytotoxic Activities. *Bioorganic Med. Chem. Lett.* **19** (2009), 1818–1821. https://doi.org/10.1016/j.bmcl.2008.12.101
21. Pillai P., Ramaswamy K. Effect of Naturally Occurring Antimicrobials and Chemical Preservatives on the Growth of *Aspergillus parasiticus*. *J. Food Sci. Technol*. **49** (2012), 228–233. https://doi.org/10.1007/s13197-011-0275-6
22. Ruberto G., Baratta M.T. Antioxidant Activity of Selected Essential Oil Components tn Two Lipid Model Systems. *Food Chem*. **69** (2000), 167–174. https://doi.org/10.1016/S0308-8146(99)00247-2
23. Kumar P., Kumaravel S., Lalitha C. Screening of Antioxidant Activity, Total Phenolics and GC-MS Study of *Vitex negundo*. *African J. Biochem. Res.* **4** (2010), 191–195. https://doi.org/10.5897/AJBR.9000213

24. Yu X., Zhao M., et al. Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a Strong Antioxidant in Glucose-histidine Maillard Reaction Products. *Food Res. Int*. **51** (2013), 397–403. https://doi.org/10.1016/j.foodres.2012.12.044
25. Justino G.C., Correia C.F., et al. Antioxidant Activity of a Catechol Derived from Abietic Acid. *J. Agric. Food Chem.* **54** (2006), 342–348. https://doi.org/10.1021/jf052062k
26. Vaquero M.J.R., Alberto M.R., Manca de Nadra M.C. Antibacterial Effect of Phenolic Compounds from Different Wines. *Food Control*. **18** (2007), 93–101. https://doi.org/10.1016/j.foodcont.2005.08.010
27. Sermakkani M., Thangapandian V. GC-MS Analysis of *Cassia italica* Leaf Methanol Extract. *Asian J. Pharm. Clin. Res*. **5** (2012), 90–94.
28. Yff B.T.S., Lindsey K.L., et al. The Pharmacological Screening of *Pentanisia prunelloides* and the Isolation of the Antibacterial Compound Palmitic Acid. *J. Ethnopharmacol.* **79** (2002), 101–107. https://doi.org/10.1016/S0378-8741(01)00380-4
29. Kubínová R., Jankovská D., Bauerova V. Antioxidant and α-glucosidase Inhibition Activities and Polyphenol Content of Five Species of *Agrimonia* genus. *Acta Fytotech. Zootech*. **2** (2012), 38–41.
30. Muruzović M.Ž, Mladenović K.G., et al. Extracts of *Agrimonia eupatoria* L. as Sources of Biologically Active Compounds and Evaluation of Their Antioxidant, Antimicrobial, and Antibiofilm Activities. *J. Food Drug Anal.* **24** (2016), 539–547. https://doi.org/10.1016/j.jfda.2016.02.007
31. Silva B.A., Ferreres F., et al. Phytochemical and Antioxidant Characterization of *Hypericum perforatum* Alcoholic Extracts. *Food Chem.* **90** (2005), 157–167. https://doi.org/10.1016/j.foodchem.2004.03.049
32. Harshaw D., Nahar L., et al. Bioactivity of *Rumex obtusifolius* (Polygonaceae). *Arch. Biol. Sci*. **62** (2010), 387–392. https://doi.org/10.2298/ABS1002387H
33. Elzaawely A.A., Tawata S. Antioxidant Capacity and Phenolic Content of *Rumex dentatus* L. Grown in Egypt. *J. Crop Sci. Biotech*. **15** (2012), 59–64. https://doi.org/10.1007/s12892-011-0063-x
34. Gawron-Gzella A., Witkowska-Banaszczak E., et al. Chemical Composition, Antioxidant and Antimicrobial Activities of *Sanguisorba officinalis* L. Extracts. *Pharm. Chem. J.* **50** (2016), 244–249. https://doi.org/10.1007/s11094-016-1431-0
35. Paudel B., Bhattarai H.D., et al. Estimation of Antioxidant, Antimicrobial Activity and Brine Shrimp Toxicity of Plants Collected from Oymyakon Region of the Republic of Sakha (Yakutia), Russia. *Biol. Res*. **47** (2014), 1–6. https://doi.org/10.1186/0717-6287-47-10
36. Sahreen S., Khan M.R., Khan R.A. Phenolic Compounds and Antioxidant Activities of *Rumex hastatus* D. Don. Leaves. *J. Med. Plants Res*. **5** (2011), 2755–2765.
37. Correia H.S., Batista M.T., Dinis T.C.P. The Activity of an Extract and Fraction of *Agrimonia eupatoria* L. Against Reactive Species. *BioFactors*. **29** (2007), 91–104. https://doi.org/10.1002/biof.552029209
38. Spiteller M., Özen T., et al. Phenolic Constituents and the *in vitro* Antioxidant Activity of the Flowers of *Hypericum venustum*. *Fitoterapia* **79** (2008), 191–193. https://doi.org/10.1016/j.fitote.2007.11.012
39. Kizil G., Kizil M., et al. Antioxidant Activities of Ethanol Extracts of *Hypericum triquetrifolium* and *Hypericum scabroides*. *Pharm. Biol*. **46** (2008), 231–242. https://doi.org/10.1080/13880200701739363
40. Dorman H.J.D., Bachmayer O., et al. Antioxidant Properties of Aqueous Extracts from Selected *Lamiaceae species* Grown in Turkey. *J. Agric. Food Chem*. **52** (2004), 762–770. https://doi.org/10.1021/jf034908v
41. Liu X., Ardo S., et al. Total Phenolic Content and DPPH. Radical Scavenging Activity of Lettuce (*Lactuca Sativa* L.) Grown in Colorado. *LWT – Food Sci. Technol*. **40** (2007), 552–557. https://doi.org/10.1016/j.lwt.2005.09.007

Մ. Մ. ԳԻՆՈՎՅԱՆ, Ն. Ժ. ՍԱՀԱԿՅԱՆ, Մ. Տ. ՊԵՏՐՈՍՅԱՆ, Ա. Հ. ԹՌՉՈՒՆՅԱՆ

ՀԱՅԱՍՏԱՆԻ ՖԼՈՐԱՅՈՒՄ ՆԵՐԿԱՅԱՑՎԱԾ ՈՐՈՇ ԴԵՂԱԲՈՒՅՍԵՐԻ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ՆԵՐՈՒԺԻ ԳՆԱՀԱՏՈՒՄԸ ԵՎ ՖԻՏՈՔԻՄԻԱԿԱՆ ԲՆՈՒԹԱԳՐՈՒՄԸ

Օքսիդային սթրեսի կողմնակի ազդեցությունները մեծագույն խնդիր են բժշկագիտության մեջ: Մի շարք իրավիճակներում մարդու օրգանիզմում օքսիդային ռեակտիվ տեսակների քանակը կարգավորելու համար անհրաժեշտ է լինում էկզոգեն հակաօքսիդիչների կիրառում: Դեղաբույսերը համարվում են նոր հակաօքսիդանտային միացությունների խոստումնալից աղբյուր: Աշխատանքի նպատակն էր գնահատել հետևյալ դեղաբույսերի՝ *Agrimonia eupatoria, Hypericum alpestre, Rumex obtusifolius* և *Sanguisorba officinalis* լուծամզվածքների հակաօքսիդային պոտենցիալը՝ օգտագործելով տարբեր քիմիական թեստեր: Բույսերի ցնդող կենսաակտիվ բաղադրիչների նույնականացման համար օգտագործվել է GC-MS մեթոդը: Ձեռք բերված տվյալներով ցույց է տրվել հետազոտված դեղաբույսերի մեծ ներուժը, որպես նոր հակաօքսիդանտային միացությունների աղբյուր:

М. М. ГИНОВЯН, Н. Ж. СААКЯН, М. Т. ПЕТРОСЯН, А. А. ТРЧУНЯН

АНТИОКСИДАНТНЫЙ ПОТЕНЦИАЛ НЕКОТОРЫХ ЛЕКАРСТВЕННЫХ РАСТЕНИЙ ФЛОРЫ АРМЕНИИ И ХАРАКТЕРИСТИКА ИХ ФИТОХИМИЧЕСКИХ ВЕЩЕСТВ

Побочное действие окислительного стресса – огромная проблема в медицине. В некоторых случаях в организме человека необходимы экзогенные антиоксиданты, чтобы регулировать количество реактивных форм. Лекарственные растения считаются многообещающим источником новых антиоксидантных соединений. Целью данного исследования было оценить антиоксидантный потенциал экстрактов следующих растении: *Agrimonia eupatoria, Hypericum alpestre, Rumex obtusifolius* и *Sanguisorba officinalis* с помощью различных химических тестов. Метод ГХ-МС использовался для идентификации летучих биоактивных компонентов растений. Результаты показали хороший потенциал протестированных растений в качестве источников новых антиоксидантных соединений.