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 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 bioactive 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. IC50 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$_2$O$_2$) reducing ability of plant extracts was evaluated according to the method described by Ruch et al. [16]. 40 mM H$_2$O$_2$ solution was prepared in 50 mM phosphate buffer (pH 7.4). H$_2$O$_2$ concentration was measured by a spectrophotometer at 230 nm wavelength. 0.2 mL of plant extract with a concentration of 1000 μg mL$^{-1}$ was added to 1.8 mL H$_2$O$_2$ 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$_2$O$_2$ 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 μg 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 μg 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 1.56×10$^5$ 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 IC₅₀ values of plant methanol extracts were determined. It was revealed that the methanol extract of *R. obtusifolius* had lowest IC₅₀ value (25.29 µg mL⁻¹ at a concentration of 0.05 mg mL⁻¹). This was nearly double the IC₅₀ 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.

| Plant name | Extract | DPPH reduction | H₂O₂ reduction | Fe²⁺-%chelation | MDA-%reduction | Total phenolic compounds, µg GAE (mg DW)⁻¹ |
|------------|---------|----------------|----------------|----------------|----------------|---------------------------------|
|            |         | % reduction in 100 µg mL⁻¹ plant crude extract | IC₅₀ value, µg mL⁻¹ | % reduction in 100 µg mL⁻¹ plant crude extract | % reduction in 225 µg mL⁻¹ plant crude extract | µg 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 | – | 348.3±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 | 209.8±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 | – | 273.8±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 | 228.9±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 μg 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 H₂O₂ 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 H₂O₂. 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 μg mL⁻¹ by reducing the number of Fe²⁺–ferrozine complexes by 37.30%, 31.75%, 73.02%, 41.27%, respectively (in the presence of 0.125 mM FeCl₂). 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 µg 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

| 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                                                                                                                                          |
| Eicosano acid                               | 45.847         | 5.16        | fatty acid                 | antimicrobial                                                                                                                                          |
| Nonadecane                                  | 46.723         | 0.72        | alkane                     | not reported                                                                                                                                          |
| 1-chloroenoic acid                          | 48.892         | 0.62        | alkane                     | not reported                                                                                                                                          |
| 1-Octadecane                                | 52.934         | 0.55        | –                          | not reported                                                                                                                                          |
| Squalene                                    | 54.237         | 0.40        | alkene                     | not reported                                                                                                                                          |
| None pure ingredients                       | 56.438         | 4.52        | triterpene                 | antibacterial, antioxidant, antitumor, pesticidal                                                                                                 |

Compounds identified in the crude methanol extract of *A. eupatoria*
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 GC-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-Pyrane-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                                               |
| Triteatracontane                                         | 29.198         | 0.68        | alkane              | insecticide                                                |
| N-[4-bromo-n-butyl]-2-Piperidinone                       | 30.260         | 0.35        | alkaloids           | antimicrobial, antioxidant, anti-inflammatory              |
| Tetrade cane                                             | 32.341         | 0.29        | alkane              | not reported                                               |
| Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimehtoxy| 33.776         | 0.27        | alkaloids           | not reported                                               |
| Çuran-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                                    | 36.427         | 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

| 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-Methyldecocane    | 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   | antimicrobial, 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,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                                              | 32.328         | 0.13        | triterpene      | antibacterial, antioxidant, antitumor, pesticidal |
| 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. Kubinová 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 μg mL$^{-1}$ resulted in 94.83% inhibition of DPPH, which is only slightly different from obtained data (98.15% reduction at a concentration 100 μg 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 μg mL$^{-1}$ and 152 μg 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 μg 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 μg 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 reactive, but it can be toxic to cells as it generates highly reactive hydroxyl radicals in cells [36]. Therefore, the neutralization of H$_2$O$_2$ 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$_2$O$_2$ reducing activity of *H. alpestre*, *R. obtusifolius* and *S. officinalis* was investigated for the first time. The literature contains data on the H$_2$O$_2$ 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$_2$O$_2$ reducing activity of the *A. eupatoria* water-ethanol extract was shown, which partly coincides with the obtained data [37]. H$_2$O$_2$ reducing activity of various species of the genus *Hypericum* has been reported. In particular, H$_2$O$_2$ 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. venustum* 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 tested 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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. Ničiforović 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. Kuliscic 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.bml.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 in 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. Kubinová 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. 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
Orchidaceae species of the prevailing species in the studied area were made to regulate the oxidative stress. Several species of the orchid family were used to control the oxidative stress in the body. These species have a high potential for use in medicine and can be used as a source of new antioxidants.

The aim of this study was to evaluate the antioxidant potential of the following plants: Agrimonia eupatoria, Hypericum alpestre, Rumex obtusifolius, Sanguisorba officinalis, and to identify their phytochemical components. The method of GC-MS was used to identify the volatile bioactive components of the plants. The results showed a good potential of the tested plants as sources of new antioxidants.