

THE CHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES  
OF SOME SPECIES OF *ARTEMISIA* GENUS, REPRESENTED  
IN ARMENIAN FLORA

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Plants are valuable sources of antioxidants, which could have beneficial effect on human health. In this respect flavonoids and other polyphenolic compounds have gained the greatest attention. The present study was undertaken to evaluate the *in vitro* antiradical activity of different extracts (ethanol, hexane, acetone, chlorophorm and methanol) of *Artemisia vulgaris* L., *A. fragrans* Willd., *A. absinthium* L. and *A. splendens* Willd., represented in Armenian flora. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to measure the radical scavenging activity of extracts obtained from *Artemisia* species. *A. fragrans* methanol and chlorophorm extracts possess the highest antiradical activity with IC<sub>50</sub> value of 87 µg/mL and 98 µg/mL, respectively. The total flavonoid content in plant extracts was determined employing AlCl<sub>3</sub> colorimetric assay. The content of total flavonoid compounds of *A. fragrans* methanol extract was 15.3±0.3 µg QE/mg. The Folin–Ciocalteu assay was used to determine the total phenolic content of studied extracts. The contents of total phenolic compounds of *A. fragrans* chlorophorm and methanol extracts were 55.9±1.7 µg and 87.3±1.8 µg of GAE/mg, respectively. In the other cases this parameter value was lower than 40 µg of GAE/mg indicated.

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**Introduction.** Medicinal plants have been used for centuries as remedies for human diseases, because they contain components of therapeutic value. Moreover, the use of plant extracts in the food, cosmetics and pharmaceutical industries is growing day by day [1, 2].

The *Artemisia* genus plant species belonging to the Asteraceae family contain products of secondary metabolism with high biological activity and are widely used in medicine, cosmetic and food industry. About 300 species of this genus are known and 16 from them are described for Armenian flora [3].

Some species of this genus have been used to treat diabetes, epilepsy, psychosis, depression, irritability, insomnia, anxiety and stress in traditional medicine. Some plants of the *Artemisia* genus showed antispasmodic, antiseptic, antibacterial, anti-malarial, antitumour, antirheumatic and hepatoprotective properties [4]. Sesquiterpene

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lactones and flavonoids are one of the most interesting ones from the pharmacological point of view. These substances are known for their reported medicinal efficacy and possess strong anti-inflammatory, antimalarial, antioxidant, antitumor, as well as immune-modulation activity. They decrease the risk of atherosclerosis, arthritis and gastrointestinal disorders [5–7].

Free radicals contribute to more than one hundred human disorders including atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and many others [8, 9]. Due to the environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals cause depletion of the immune system antioxidants, and even the change in gene expression. They cause severe oxidative damage of proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation with subsequent tissue injury [10, 11]. The oxidation process also is one of the most important routes for producing free radicals in food and medicinal preparations [12, 13].

Antioxidants possess the ability of protecting organisms from damages caused by free radical-induced oxidative stress. Many medicinal plants contain large amounts of antioxidants such as polyphenols, vitamins, selenium,  $\beta$ -carotene, lycopene, lutein and other carotenoids, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [14]. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been extensively added to foodstuffs, although their use has begun to be questioned, because of their toxicity, so there is considerable interest to them for the development of natural preparations with antioxidant properties [15–18].

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or pathogen aggressions. Polyphenols are responsible for the bitterness, astringency, color, flavor, odor. In the last decade, there has been much interest to the potential health benefits of dietary plant polyphenols as antioxidant. Epidemiological studies and meta-analyses strongly suggest the long-term consumption of plants rich in polyphenols in order to combat against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases [19, 20]. More than 8000 polyphenolic compounds have been identified in various plant species. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Polyphenols main classes include phenolic acids, flavonoids, stilbenes and lignans [21, 22].

The investigation of polyphenolic composition of medicinally valuable plant species is also an important issue, which may contribute to the improvement of conventional food with added health benefits [23].

The aim of this study was to investigate the phenolic and flavonoids composition as well as antioxidant capacity of *A. vulgaris*, *A. fragrans*, *A. absinthium* and *A. splendens* extracts.

### Materials and Methods.

**Plant Material.** The investigated plants (*A. vulgaris* L., *A. fragrans* Willd., *A. absinthium* L. and *A. splendens* Willd.) were collected from Aragatsotn Region (Armenia, v. Mughni, 1500–1600 m a.s. level, N 40°22.085', E 44°22.815') during the flowering period.

**Extract Preparation.** Plant material was dried at 60°C. 1 g powdered dried plant material was homogenized in 10–15 mL solvent (ethanol, hexane, methanol, acetone and chlorophorm) and left overnight at ~10°C. Extracts were centrifuged for 5 min at 5000 rpm, and the supernatants were isolated. The precipitates were extracted by 4-folds, and the combined supernatants were dried by evaporation at room temperature. The evaporated mass was solved in ethanol, and the extracts in different dilutions were used.

**Determination of Radical Scavenging Activity.** Scavenging free radical potentials were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Catechin was used as standard. Sample solution contained 125 µL (1 mM) DPPH, 375 µL ethanol and 500 µL of test-solution (extract and catechin with different concentrations (1000 µg/mL, 500 µg/mL, 100 µg/mL, 50 µg/mL and 10 µg/mL, respectively). Test-solution was replaced by ethanol in the control sample. The absorbance was measured at the wavelength of 517 nm using spectrophotometer Genesys 10S UV-Vis (“Thermo Scientific”, USA).

The radical scavenging activity was calculated using the following formula:

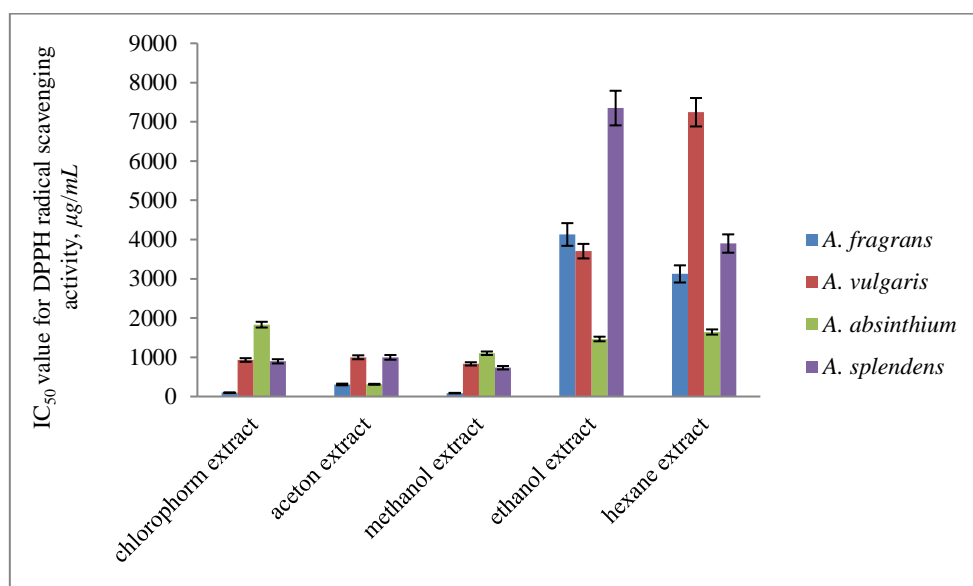
$$\text{Radical scavenging activity (\%)} = (A_c - A_s) / A_c \times 100,$$

where  $A_c$  is absorbance of control (DPPH without the addition of test solution),  $A_s$  is sample absorbance.  $IC_{50}$  calculated denote the concentration of investigated samples required to decrease the DPPH absorbance at 517 nm by 50% [23, 24].

**Determination of Total Phenolic Content.** The concentration of phenolics in plant extracts was determined using Folin–Ciocalteu assay. The reaction mixture consists of 0.5 mL of extract (1 mg/mL) and 0.1 mL of Folin–Ciocalteu reagent. After 5 min, 1 mL of 7% sodium carbonate ( $Na_2CO_3$ ) was added. The volume was made up to 2.5 mL by adding distilled water. A set of standard solutions of gallic acid (5–100 mg/mL) were prepared in the same manner as described earlier. The mixtures were incubated for 90 min at room temperature and the absorbance for test and standard solutions was determined against the reagent blank at 765 nm with an Genesys 10S UV-Vis spectrophotometer. Total phenolic content was expressed as µg of GAE/mg of extract [25, 26].

**Determination of Total Flavonoid Content.** The total flavonoid content in plant extracts was determined employing  $AlCl_3$  colorimetric assay. The extract was dissolved in 80% ethanol to obtain a final concentration of 1 mg/mL. 0.5 mL of this extract solution was mixed with 0.1 mL of  $AlCl_3$  (10%), 0.1 mL of sodium acetate (1 M) and 2.8 mL of distilled water. The sample was incubated for 15 min and the absorbance of the samples was measured at 415 nm against a blank consisting of distilled water utilizing a Genesys 10S UV-Vis spectrophotometer. Total flavonoid content was determined employing a calibration curve of quercetin (Q), as a reference flavonoid (0–1000 µg/mL) and results were expressed in terms of Q equivalents (QE) per g extract dry weight [27].

**Results and Discussion.** DPPH assay is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The antiradical activity of *A. vulgaris*, *A. fragrans*, *A. vabsinthium* and *A. splendens* extracts is expressed in Figure (data were expressed as  $IC_{50}$ ). The  $IC_{50}$  value for the positive control was determined as  $13.08 \mu\text{g/mL}$  ( $R^2 = 0.93$ ) (data not shown). The highest antiradical activity possess the *A. fragrans* methanol ( $IC_{50}=87 \mu\text{g/mL}$ ,  $R^2 = 0.98$ ) and chlorophorm ( $IC_{50}=98 \mu\text{g/mL}$ ,  $R^2 = 0.94$ ) (data not shown) extracts.



The radical scavenging activity of chlorophorm, acetone, methanol, ethanol and hexane extracts of *A. vulgaris*, *A. fragrans*, *A. absinthium* and *A. splendens*.

The results of total phenolic content of *A. vulgaris*, *A. fragrans*, *A. absinthium* and *A. splendens* extracts expressed as  $\mu\text{g}$  of GAE/mg (Tab. 1) (the standard curve equation:  $y=0.009x + 0.148$ ,  $R^2 = 0.992$ ) (data not shown). Methanol extract had the highest quantity of the phenolic compounds ( $87.3 \pm 1.8 \mu\text{g}$  of GAE/mg) followed by chlorophorm ( $55.9 \pm 1.7 \mu\text{g}$  of GAE/mg) and acetone ( $38.1 \pm 1.1 \mu\text{g}$  of GAE/mg) extracts.

Table 1

Total phenolic contents of chlorophorm, acetone and methanol extracts of *A. vulgaris*, *A. fragrans*, *A. absinthium*, *A. splendens*, expressed in terms of gallic acid equivalent ( $\mu\text{g}$  of GAE/mg of extract)

| Extract     | Total phenolic content, $\mu\text{g}$ of GAE/mg |                    |                      |                     |
|-------------|---|--------------------|----------------------|---------------------|
|             | <i>A. fragrans</i>                              | <i>A. vulgaris</i> | <i>A. absinthium</i> | <i>A. splendens</i> |
| Chlorophorm | $55.9 \pm 1.7$                                  | $9.0 \pm 0.3$      | $12.9 \pm 0.4$       | $13.4 \pm 0.4$      |
| Acetone     | $38.1 \pm 1.1$                                  | $41.3 \pm 1.2$     | $12.6 \pm 0.4$       | $22.8 \pm 0.7$      |
| Methanol    | $87.3 \pm 1.8$                                  | $37.9 \pm 0.8$     | $4.0 \pm 0.1$        | $81.8 \pm 1.6$      |

In the other cases this parameter value was lower than  $40 \mu\text{g}$  of GAE/mg. The radical scavenging activity value of investigates extracts was in strong correlation with the total phenolic content (see below).

The results of total flavonoids content of *A. vulgaris*, *A. fragrans*, *A. absinthium* and *A. splendens* extracts expressed as  $\mu\text{g QE/mg}$  (Tab. 2) (the standard curve equation: (Tab. 1)  $y=0.0024x+0.0416$ ,  $R^2=0.99$ ) (data not shown). Methanol extract had the highest ( $15.3\pm 0.7 \mu\text{g QE/mg}$ ) quantity of flavonoid compounds followed by chlorophorm ( $5.7\pm 0.3 \mu\text{g QE/mg}$ ) and acetone ( $4.1\pm 0.1 \mu\text{g QE/mg}$ ) extracts.

In the other cases this parameter value was lower than  $4 \mu\text{g QE/mg}$ . Obtained data indicated that there are also some correlations between the antiradical activity and total flavonoids content of investigated plants extracts.

Table 2

The total flavonoid contents of chlorophorm, acetone and methanol extracts of *A. vulgaris*, *A. fragrans*, *A. absinthium*, *A. splendens*, expressed in terms of quercetin equivalent

| Extract     | Total flavonoid content, $\mu\text{g QE/mg}$ |                    |                      |                     |
|-------------|--|--------------------|----------------------|---------------------|
|             | <i>A. fragrans</i>                           | <i>A. vulgaris</i> | <i>A. absinthium</i> | <i>A. splendens</i> |
| Chlorophorm | $5.7\pm 0.13$                                | $0.2\pm 0.001$     | $3.7\pm 0.02$        | $1.4\pm 0.01$       |
| Acetone     | $4.1\pm 0.1$                                 | $3.9\pm 0.2$       | $3.95\pm 0.02$       | $1.8\pm 0.01$       |
| Methanol    | $15.3\pm 0.3$                                | $3.9\pm 0.22$      | $0.012\pm 0.001$     | $3.4\pm 0.02$       |

Our investigations showed that the extracts obtained from *A. vulgaris*, *A. fragrans*, *A. absinthium* and *A. splendens* possess significant DPPH free radical scavenging ability. Moreover, this activity depends on the type of solvent used for extraction. It is interesting to note that it is observed that the highest antiradical activities of all plants were expressed in acetone, chloroform and methanol extracts. Whereas the hexane and ethanol extracts express low activity. This data can be explained by the solubility features of biochemicals, synthesized in plants.

The total concentration of phenols and flavonoids in *A. vulgaris*, *A. fragrans*, *A. absinthium*, *A. splendens* was high in acetone, chloroform and methanol extracts. The rather high total phenols and flavonoids content in all plants explains the high antiradical and antioxidant activity. The potential of these plants should be further evaluated for application in modern medicine, food industry and cosmetics due to their naturally high content of substances with antioxidant activity.

**Conclusion.** Based on our investigation data, it was possible to conclude that the *A. fragrans* chlorophorm and methanol extracts possess the highest antiradical activity, whereas the others have moderate (in case of acetone extract) or low activity. The overall results indicate that the *A. fragrans* contain the highest content of polyphenols and flavonoid. Thus, we can offer some plants of the *Artemisia* genus growing in Armenia as a possible source of preservatives in food processing, as well as an alternative source of antioxidant rich substances for the development of cosmetics and medicinal preparations.

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ՀԱՅԱՍՏԱՆՈՒՄ ԱՃՈՂ *ARTEMISIA* ՑԵՂԻ ՈՐՈՇ ՏԵՍԱԿՆԵՐԻ  
ՔԻՄԻԱԿԱՆ ԿԱԶՄԸ ԵՎ ՀԱԿԱՕՔՄԻԴԱՆՏԱՑԻՆ ԱԿՏԻՎՈՒԹՅՈՒՆԸ

Բույսերը հակաօքսիդանտային նյութերի արժեքավոր աղբյուր են և կարող են բարենպաստ ազդեցություն ունենալ մարդու առողջության վրա: Այս առումով մեծ ուշադրություն է դարձվում ֆլավոնոիդներին և այլ պոլիֆենոլային միացություններին: Ներկայացված աշխատանքում ուսումնասիրվել է Հայաստանում աճող *Artemisia vulgaris* L., *A. fragrans* Willd., *A. absinthium* L. և *A. splendens* Willd. բուսատեսակների տարբեր լուծամրգավածքների (էթանոլային, հեքսանային, ացետոնային, քլորոֆորմային և մեթանոլային) *in vitro* հակառադիկալային ակտիվությունը: *Artemisia* ցեղի տարբեր տեսակներից ստացված լուծամրգավածքների հակառադիկալային ակտիվությունը գնահատելու համար օգտագործվել է 1,1-դիֆենիլ-2-պիկրիլիդրազիլը: Ամենաբարձր հակառադիկալային ակտիվություն ունեն *A. fragrans* բույսի մեթանոլային և քլորոֆորմային լուծամրգավածքները ( $IC_{50}$ -ի արժեքներն են՝ 87 և 98 մկգ/մլ, համապատասխանաբար): Բուսական լուծամրգավածքներում ֆլավոնոիդների ընդհանուր պարունակությունը որոշվել է  $AlCl_3$  գունաչափական

վերլուծության միջոցով: *A. fragrans*-ի մեթանոլային լուծամզվածքում ընդհանուր ֆլավոնոիդային միացությունների պարունակությունը կազմել է  $15,3 \pm 0,3$  մկգ ԿՀ/մգ: Ուսումնասիրված լուծամզվածքների ընդհանուր ֆենոլային միացությունների պարունակությունը որոշելու համար օգտագործվել է Ֆոլին–Չեոկատեուի մեթոդը: Ընդհանուր ֆենոլային միացությունների պարունակությունը *A. fragrans* բույսի քլորոֆորմային և մեթանոլային լուծամզվածքներում կազմել է  $55,9 \pm 1,7$  և  $87,3 \pm 1,8$  մկգ ԳԹՀ/մգ, համապատասխանաբար: Մյուս դեպքերում այս պարամետրի արժեքը ցածր էր 40 մկգ ԳԹՀ/մգ-ից:

А. М. БАБАЯН, М. Т. ПЕТРОСЯН, Н. Ж. СААКЯН

#### АНТИРАДИКАЛЬНАЯ АКТИВНОСТЬ И ХИМИЧЕСКИЙ СОСТАВ НЕКОТОРЫХ ВИДОВ РОДА *ARTEMISIA*, ПРЕДСТАВЛЕННЫХ ВО ФЛОРЕ АРМЕНИИ

Растения являются ценными источниками антиоксидантов, которые могут оказать благотворное влияние на здоровье человека. В связи с этим наибольшее внимание уделяется флавоноидам и другим полифенольным соединениям. Настоящее исследование проведено с целью оценки антирадикальной активности разных экстрактов (этанольный, гексановый, ацетоновый, хлороформный и метанольный) *Artemisia vulgaris* L., *A. fragrans* Willd., *A. absinthium* L. и *A. splendens* Willd., представленных в армянской флоре. Для определения антирадикальной активности экстрактов, полученных из различных видов *Artemisia*, использовали 1,1-дифенил-2-пикрилгидразил. Метанольный и хлороформный экстракты *A. fragrans* обладали самой высокой антирадикальной активностью со значением  $IC_{50}$ , равным 87 и 98 мкг/мл соответственно. Общее содержание флавоноидов в растительных экстрактах определяли с помощью колориметрического анализа с использованием  $AlCl_3$ . Содержание общих флавоноидных соединений в метанольном экстракте *A. fragrans* составило  $15,3 \pm 0,3$  мкг КЭ/мг. Методом Фолина–Чокальтеу определяли общее содержание фенолов в исследуемых экстрактах. Содержание общих фенольных соединений в хлороформном и метанольном экстрактах *A. fragrans* составило  $55,9 \pm 1,7$  и  $87,3 \pm 1,8$  мкг ГАЕ/мг, соответственно. В остальных случаях значение этого параметра было ниже 40 мкг ГАЭ/мг.