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THE RADICAL SCAVENGING ACTIVITY 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. Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases. 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 subspecies aerial parts. Catechin was used as a positive reference. Different concentrations of extracts were used and results were expressed with IC_{50} values.

Artemisia vulgaris, A. fragrans, A. absinthium, *A. splendens*, DPPH, antiradical activity.

The *Artemisia* genus plants species belonging to the Asteraceae family which contain secondary metabolic products with high biological activity are widely used in medicine, cosmetic and food industry. About 300 species of this genus are knownand 16 from them are described for Armenian flora.

These herbs have been used worldwide in folk medicine since ancient times. They have been used as tonics, antimalarials, antihelmintics, and antidiabetics, and in treating wounds, bronchitis, ulcers, and tuberculosis [1]. There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial, and antifungal activities of different Artemisia species [2]. The chemical studies on Artemisia species indicate that all classes of compounds are present in the genus with particular reference to terpenoids and flavonoids. The rich accumulation of essential oils and other terpenoids in the genus is responsible for using of various species for flavouring foods or liqueurs [3,4].

The active components of these plants include flavonoids, coumarins, sesquiterpene lactones, volatile oils, inulin, and traces of alkaloids. The chief compounds of volatile oils include camphor, camphene, 5-thujone, germacrene D, 1,8-cineole, and C-caryophyllene [5].

Changing environmental conditions are giving rise to a variety of free radicals, which plants have to deal with them in order to survive. Reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals [6]. Free radicals have been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation, giving rise to studies of antioxidants for the prevention and treatment of diseases. The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders [7]. Medicinal plants are therefore being investigated for their antioxidant properties, and the demand for natural antioxidants and food presservatives is increasing [8].

The aim of our investigation was to study the dependence of radical scavenging activity on *A. vulgaris, A. fragrans, A. absinthium*, *A. splendens* extraction conditions.

Material 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, Mughni, 1500-1600 *m* above sea level, N 40° 22.085′, E 44° 22.815′) during the flowering period.

Extract preparation. Plant material was dried at 60°C for 24h. 1 g powdered dried plant material was homogenized in 10 to 15 mL solvent (ethanol, hexane, methanol, aceton and chlorophorm) and left overnight at ~10°C. Extract was centrifuged for 5 minutes at 5000 rpm, and the supernatant was isolated. The precipitate was extracted by 4 - folds, and the combined supernatant was 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 in ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [9]. Catechin was used as standard. Sample solution contained 125 μL (1mM) 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/mlrespectively)). Test-solution was replaced by ethanol in the control sample. The absorbance was measured at the wave length of 517 nm using spectrophotometer Genesys 10S UV-Vis (Thermo Scientific USA).

The radical scavenging activity was calculated using the following formula:

Radical scavenging activity (%) = $A_c - A_s / A_c$ x 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%.

Results and discussion

DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The DPPH assay indicated the antiradical activity of *A. vulgaris* L., *A. fragrans* Willd.,*A. absinthium* L. and *A. splendens* Willd. extracts; the antiradical properties of the studied samples are expressed in figure 1 and figure 2 (data were express as IC_{50} – the extract concentration which neutralize the 50 % of DPPH free radicals). The scavenging effect (IC_{50} value) was in the following order: catechin (standard) (IC_{50} 13.08 µg/mL (R^2 = 0.93)) (data not shown), *A. vulgaris* methanol (IC_{50} 832 µg/ml (R^2 = 0.85)), chlorophorm (IC_{50} 931 µg/ml (R^2 = 0.96)), acetone (IC_{50} 1000 µg/ml (R^2 = 0.93)),ethanol (IC_{50} 7407 µg/ml (R^2 = 0.99)) and hexane (IC_{50} 48077 µg/ml (R^2 = 0.99)) extracts; *A. fragrans* methanol (IC_{50} 87 µg/ml (R^2 = 0.68)), chlorophorm (IC_{50} 98 µg/ml (R^2 = 0.84)), acetone (IC_{50} 307 µg/ml (R^2 = 0.85)), hexane (IC_{50} 13889 µg/ml (R^2 = 0.98)) and ethanol (IC_{50} 16129 µg/ml (R^2 = 0.78)) extracts; *A. absinthium* acetone (IC_{50} 311 µg/ml(R^2 = 0.84)), methanol (IC_{50} 1103 µg/ml (R^2 = 0.92)), ethanol (IC_{50} 1466 µg/ml (R^2 = 0.99)), hexane (IC_{50} 1664 µg/ml (R^2 = 0.99)) and chlorophorm (IC_{50} 1831 µg/ml (R^2 = 0.99)) extracts; *A. splendens* methanol (IC_{50} 734 µg/ml (R^2 = 0.89)), chlorophorm (IC_{50} 897 µg/ml (R^2 = 0.93)), acetone (IC_{50} 998 µg/ml (R^2 = 0.96)), hexane (IC_{50} 24509 µg/ml (R^2 = 0.91)) and ethanol (IC_{50} 125000 µg/ml (R^2 = 0.78)) extracts.

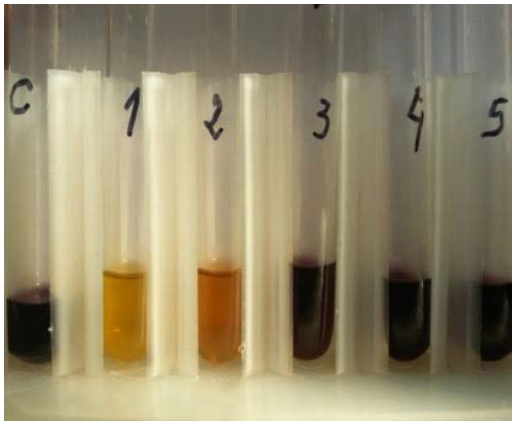

Fig. 1.The DPPH scavenging activity of *Artemisia fragrans* acetone extract 1.0; 0.5; 0.1; 0.05; 0,01 mg/ml 1-5,C – control.

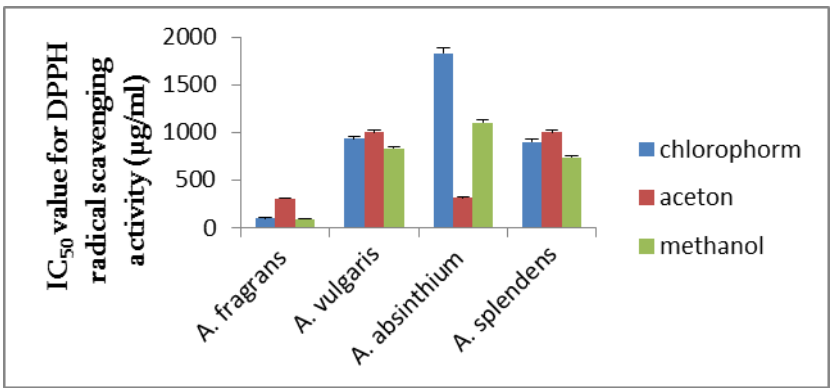

Fig. 2.The radical scavenging activity of chlorophorm, acetone and methanol extracts of *A. vulgaris, A. fragrans, A. absinthium*, *A. splendens*, expressed with IC_{50}.

The radical scavenging activity value of investigates extracts was correlated with the total phenolic content (data not shown).

The results show that hexane and ethanol extracts did not express any radical scavenging activity, so the further investigations were carried out with the most active fractions.

Conclusions

According to our investigation data it was possible to conclude that the highest antiradical activity possess the A. fragrans chlorophorm and methanol extracts, whereas the others have moderate (in case of acetone extract) or low activity.

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