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Investigation of radical scavenging potential of some populations of *Artemisia spicigera* in relation to their flavonoid content

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Abstract—**Imbalanced production and consumption of reactive oxygen species, leading to oxidative stress, is implicated in the pathophysiology of a plethora of genetic and acquired disorders, such as cancer, arteriosclerosis, malaria and rheumatoid arthritis, as well as neurodegenerative diseases and ageing processes. The protective effects of plants have long been attributed to their antioxidant compounds, such as polyphenols, carotenoids, and vitamins C and E. The purpose of this study was comparing antioxidative potential of several population of *Artemisia spicigera* (Asteraceae), collected from Ahar (ASA), Khaje (ASK), Dare-diz (ASD), Sufian (ASS) and Julfa (ASJ) in East Azarbayjan province.**

In the present study the variation of antioxidant activity and radical scavenging potential of methanolic extracts from the aerial parts of several population of *Artemisia spicigera* was investigated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging property. The amount of total phenolics and total flavonoid contents were determined by spectrophotometric methods.

In order to quantify the antioxidant activity, the IC_{50} was calculated after 5 minute. Compounds ASK, ASA, ASS, ASD and ASJ significantly showed DPPH scavenging activity with IC_{50} values of 58.93, 29.74, 32.18, 62.94 and 64.18, respectively. Sample ASA showed the highest antioxidant and radical scavenging potential.

In conclusion there were significant difference in phenolic content and antioxidant activity among various populations of *Artemisia spicigera*.

Keywords: Antioxidant activity, Artemisia spicigera, DPPH, flavonoid and phenolic compounds.

I. INTRODUCTION:

Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Natural antioxidant compounds have pharmacological potent with low or no side effects for use in preventive medicine and the food industry. Plants are the source of antioxidants, which prevent the oxidative stress. Oxidative stress caused by the reduction process of oxygen to reactive oxygen species (ROS). High levels of ROS cause damage to cells, and have an important role in human diseases, such as cancer, neurological degeneration, and arthritis, also they involves in the process of aging. Therefore, compounds with antioxidant properties may help prevent many ROS-related diseases [1]. The most important classes of natural antioxidants include tocopherols, flavonoids and phenolic acids, which are found in most plant sources. Several flavonoids are reported as scavengers of free radicals and Phenolic compounds are one of the major groups of natural antioxidants [2, 3].

Researchers have paid particular attention to the medicinal plants. The genus Artemisia L. includes 15 perennial aromatic herbs and shrubs that grow wild in dry or semi-dry habitats and it is one of the largest and most widely distributed of the Astraceae (Compositae) in the world [4]. Members of this genus have a distinctive scent or taste and are used in the liqueur-making industry [5]. Among them *A.spicigera* are found growing naturally in different areas of East-Azarbayjan in Iran. Phytochemical analysis of *A. spicigera* shows that it is a rich source of 1,8-cineole and terpinen-4-ol and because of these compounds it have inhibitory effects on the growth of bacteria, yeasts and fungi. Also previous studies prove the ability of *A. Spicigera* in scavenging free radicals [4, 5, 6]. Previous studies showed the high ability of *A.Spicigera* in deactivation of oxidants [6, 7].

The aim of this study was to compare the antioxidant ability of the extracts isolated from the aerial parts of the several population of *A. Spicigera* that are collected from various ecological areas.

II. MATERIALS AND METHODS:

A. Chemicals

1,1-Diphenyl- 2- picrydrazyl (DPPH) and Quercetine were purchased from Sigma chemical company (Steinheim, Germany). Folin– Ciocalteu's reagent was acquired from Merck company (Germany). All other reagents were of analytical reagent (AR) grade.

B. Plant materials and preparation of extracts

Aerial parts (stems, leaves, flowers, and fruits) of several population of *A.spicigera* , growing wild in Iran, were collected in summer from Ahar (ASA), Khaje (ASK), Dare-diz (ASD), Sufian (ASS) and Julfa (ASJ) in northwestern Iran. The plant was identified by A.Taleb pour from Faculty of Agriculture, University of Tabriz. The plant samples were chopped in the field and air-dried in the laboratory at room temperature in the shade, and then powdered. Dried powder of aerial parts of *A.spicigera* was extracted with methanol at 25°C twice for 24h. The solvent was evaporated in vacuum, and dried extracts were stored at 20°C until use [8].

C. Antioxidant assays

Each sample was accurately weighted and macerated in DMSO at a concentration 1mg/mL (or 2mg/ml for DPPH assay) and series of diluted crude extract solutions was prepared for antioxidant assays.

1). DPPH radical scavenging capacity

Antioxidant potential was assessed according to the reports of Shimada, Fujikawa, Yahara, and Nakamura (1992) and Epsin, Soler-Rivas, and Wichers (2000). The main concentration of samples is 2 mg/ml. An aliquot of each sample (10, 30, 50, 70 and 100 µg/ml) was mixed with 0.1 mM DPPH (prepared with MtOH) to prepare the final volume 2 mg/ml and incubated for 30 min. The absorbance (Abs) was read at 517 nm. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = [$Abs_{control}$ _ Abs_{sample}] / $Abs_{control}$ × 100.

To quantified the results IC50 value, the effective concentration that could scavenge 50% of the DPPH radicals, were calculated [9].

D. Total phenol contents determination

The Phenolic contents of extracts were determined by using a colorimetric method as described by Shimadzu, Kyoto, Japan using Folin–Ciocalteau's phenol reagent. 0.1 ml of samples were mixed with 2.0 ml of Na_2CO_3 (2% W/V) and allowed to stand for 2 min at room temperature. After addition of 0.1 ml of Folin–Ciocalteau's phenol reagent (50%), the reaction mixture was mixed thoroughly and it remained at room temperature for 30 min at room temperature in the dark. Then incubated, absorption of the samples was measured at 720 nm against the different concentration of Quercetin (0.125M, 0.25M, 0.5M, 1M) as standards [10].

E. Estimation of the total flavonoid contents

An aluminium chloride colourimetric method was used for flavonoids determination reported by Kaijv, Sheng, and Chao (2006). 0.15 ml of aluminium chloride 10%, 75 ml of $NaNO_2$ (5% W/V) and 0.5 ml NaoH (1 mol/L) were aadded to each extract (0.25 ml) and distilled water was used to make the final volume 2.5 ml. And allowed to stand at room temperature for 5 min; the absorbance of the reaction mixture was measured at 507 nm with UV Spectrophotometer. Absorbance of the samples was compared with blank solution [11].

III. RESULTS AND DISCUSSION:

The DPPH scavenging test was selected since it is widely used and simple. The Folin-Ciocalteu, used to measure the total phenolic compounds and determination of total flavonoids was done because of the importance of flavonoids as a natural antioxidants.

A. Antioxidant activity (DPPH assay)

In this method the antioxidants react with the stable free radical, a,a-diphenyl-b-picrylhydrazyl (with violet colour) and convert it to a,a-diphenyl-b-picrylhydrazine with discoloration. the extent of the reaction will depend on the hydrogen donating ability of the antioxidants and the degree of discoloration indicates the scavenging potential of the samples[12,13]. The radical scavenging activity of the populations of *A.spicigera* extracts were determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radical. Positive DPPH test suggests that the samples are free radical scavengers. The radical scavenging percentages of samples are calculated against the blank solution and samples showed various results that are presented in figure 1 and 2. Among the determined samples, ASA showed the highest scavenger property. In order to quantify the antioxidant activity, the IC50, concentration of sample that scavenges 50% of free radicals, was further calculated and is shown in Table 1. Sample ASA had the lowest IC_{50} value [8].

B. Determination of total phenolic contents

Under alkaline conditions, Folin–Ciocalteu's (FC) phenol reagent (yellow colour) reacts with phenolic compounds and formed a phenolate anion by dissociation of a phenolic

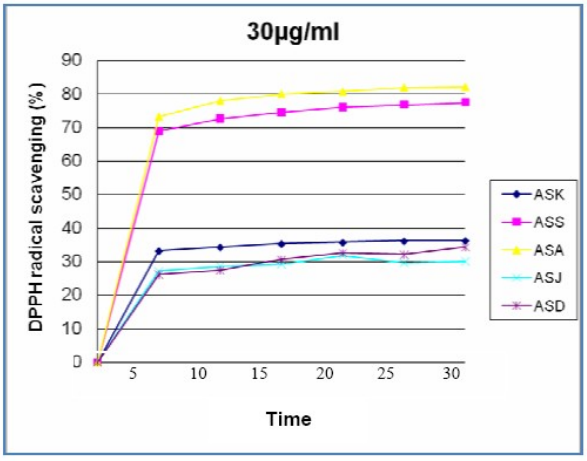

Figure 1. Kinetic behavior of radical scavenging activity of aerial parts extracts from A.spicigera. DPPH dissolved in methanol to a concentration of 0.1 mM was reacted with 30 µg/mL of extracts.

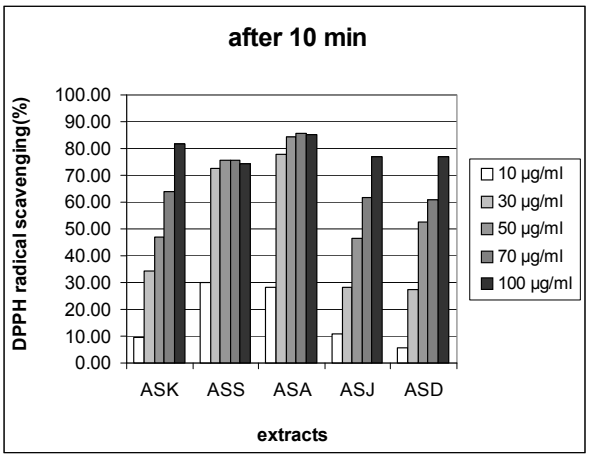

Figure 2. Radical scavenging activity of various concentrations of the samples. The kinetics of scavenging effects was determined in 10 min.

TABLE I. AMOUNTS OF EXTRACTS NEEDED TO SCAVENGE 50% OF DIPHENYLPICRYLHYDRAZYL FREE RADICAL IN 5 MIN.

Extracts	IC_{50} (µg Ext/ml)
ASK	58.93
ASA	29.74
ASS	32.18
ASD	62.94
ASJ	64.18

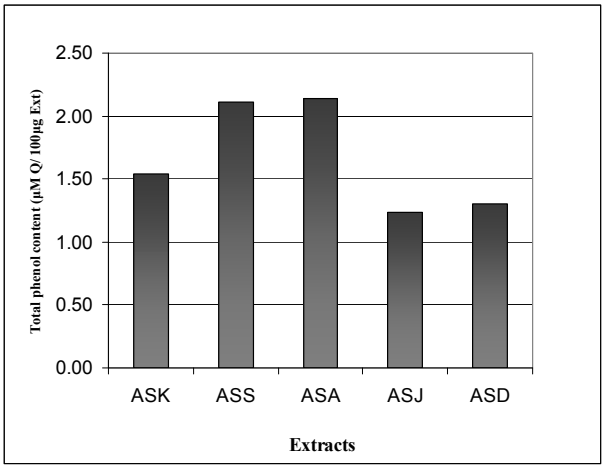

Figure 3. Total phenolic content (TPC) of various extracts from A.spicigera. Results are expressed as micromole quercetin equivalents per gram of extract (µMQ=100µg extract).

hydrogen atom. This sequence of reversible one- or two-electron reduction reactions leads to blue-coloured chromophores being formed between phenolate and the FC reagent [2]. According to these findings the total phenolics content of several populations of *A.spicigera* were measured as µM Quercetine/100µg extracts and the results is showen in figure 3. There are many papers that reports excellent linear correlations between ‘‘total phenolic profiles” and ‘‘antioxidant capacity”. Also in this study sample ASA which showed the highest antioxidant activity, have the higher phenolic contents.

C. Measuring total flavonoid contents

Flavonoids are a class of secondary plant phenolics, which act as pharmacological active compounds in many medicinal plants with their powerful antioxidant properties [15]. Flavonoids have the basic skeleton of diphenylpropanes (C6–C3–C6) with various oxidation level of the central pyran ring; they could provide strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions [14]. These compounds interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes [15].In this study we measured total flavonoid contents of five population of *A.spicigera* and showed the achieved data in the figure 4. According to this figure, samples ASS and ASA have the higher flavonoid contents. It shows a significant relation between the flavonoid contents of the extracts and their antioxidant ability.

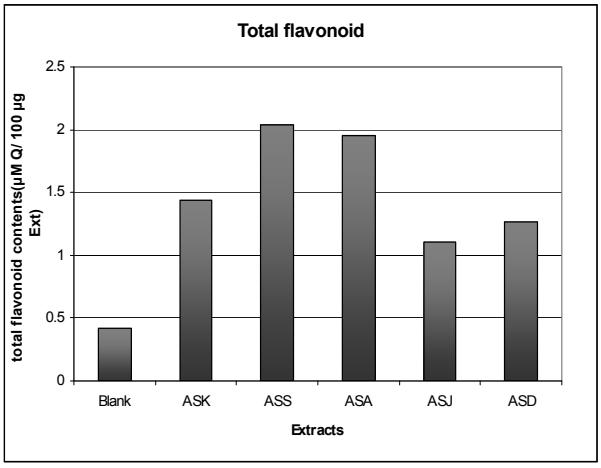

Figure 4. Total flavonoid content (TFC) of aerial parts extracts from A.spicigera. Results are expressed as micromole quercetin equivalents per gram of extract (µMQ=100µg extract).

IV. CONCLUSION:

Results showed that sample ASA has the highest amount of flavonoid and phenol contents and is the most powerful free radical scavenger. Also it has the lowest IC_{50} in DPPH assay (29.74). In addition, relation of the TPC and TFC with

radical scavenging activity of the samples is shown in figure 5 and 6.

Taken collectively, these results conclude that populations of *A*.spicigera extracts show variable antioxidant activity. Difference of the antioxidant potential of several populations of *A.spicigera* extracts shown in the current study indicates that ecological conditions of the growth area have the important role in amount of antioxidant ability of the plants.

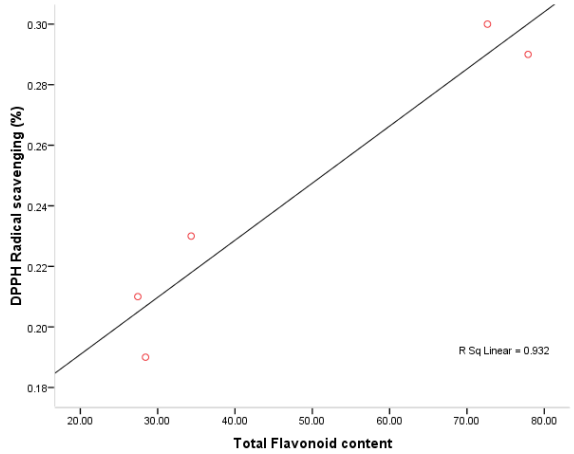

Figure 5. Correlation between A.spicigera organic extracts DPPH scavenging activity and their total Flavonoid contents.

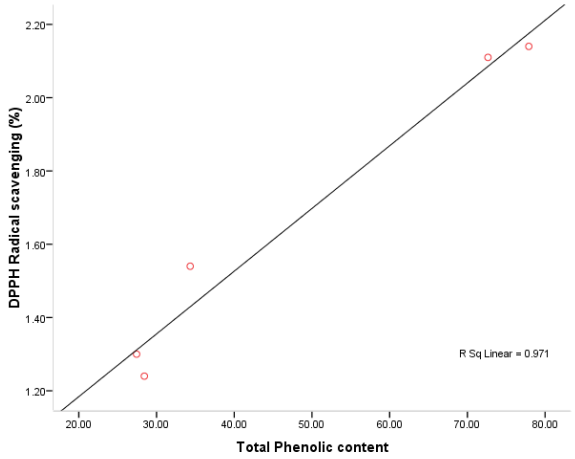

Figure 6. Correlation between A.spicigera organic extracts DPPH scavenging activity and their total phenolic contents.

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