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Comparative study of ABTS radical scavenging activity and flavonoid contents in several populations of *Teucrium polium*

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***Abstract*—Reactive oxygen species play a critical role in cardiovascular diseases, inflammatory diseases, neurodegenerative disorders, cancer and aging. The objective of this study was to compare the in vitro variation of antioxidative and radical scavenging potential of methanolic extracts from the aerial parts of four populations of *Teucrium polium* (Lamiaceae) which are collected from Bostan abad (TPB), Pole azadegan (TPP), Shurestan (TPS), Makidy (TPM).**

In order to quantify the antioxidant activity, extracts were evaluated for ABTS [2, 20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] scavenging property. The content of total phenols and total flavonoids in cited populations was determined by using UV/Vis spectrophotometric methods.

In all assays sample TPB show the highest ABTS radical scavenging activity. This sample also is rich in phenolic and flavonoid contents.

Significant differences were found in phenolic and flavonoid content and antioxidant activity among various populations of *Teucrium polium*.

Key words: Antioxidant activity, Teucrium polium, ABTS, total flavonoid.

I. INTRODUCTION:

Oxidative stress refers to an imbalance between the production of free radicals and the antioxidant defense system. Reactive oxygen species (ROS) are various forms of activated oxygen which causes oxidative damage. Mechanisms responsible for the ROS-mediated injuries mainly include lipid peroxidation, oxidative DNA damage and protein oxidation [1,2]. Antioxidants are compounds that detoxify ROS and prevent their damage through multi-mechanisms. Synthetic antioxidants have been in use as food additives for a long time, but reports on their involvement in chronic diseases have restricted their use in foods. Therefore, international attention has been focused on natural antioxidants mainly from plant sources[3,4].

Teucrium polium which belongs to the Lamiaceae, is one of the wild-growing flowering species and is found abundantly in Iran. The biological activities of *T.polium* such as anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive, hypolipidemic, anti-rheumatoid, and hypoglycemic affects are widely reported. There are also some reports in the literature for antioxidant affects of crude extract of *T.polium*[1].

The objective of this study was to investigate the antioxidant potencies of four populations of *Teucrium polium* which are collected from different places around East Azarbaijan, Iran. To compare the antioxdant activity we used ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium) radical scavenging assay which is applicable for both lipophilic and hydrophilic antioxidants. Total phenol and total flavonoid content in extracts, which reason antioxidant and free radical scavenging activity, was determined spectrophotometrically.

II. MATERIALS AND METHODS:

A.Materials

ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium) and Folin–Ciocalteu's reagent was obtained from Merck (Germany). (Dimethyl sulfoxide) DMSO was obtained from Fluka (Buchs, Switzerland).All other reagents were of analytical reagent (AR) grade.

B. Plant material

Four different populations of *Teucrium polium* include TPB, TPS, TPP and TPM were collected from Bostan Abad, Shorestan, Pole Azadegan and Makidy from East Azarbaijan, Iran during summer. Samples were approved by A.Taleb pour from Faculty of Agriculture, University of Tabriz. The plants aerial parts were air dried, protected from direct sunlight, and then powdered. The powder was kept in a closed container at 8°C.

C. Preparation of T.polium extracts

The powdered plant material (160 gr) was extracted with methanol (MtOH) (90%), at room temprature (RT) overnight. The extraction was repeated three-times and the solvent was evaporated in vacuum, and dried extracts were stored at 20°C until use. All the extracts obtained were used in antioxidant measurements and determination of total phenol and flavonoid contents.[3]

D.ABTS radical scavenging assay

The ABTS assay is based on the ability of the antioxidants to scavenge the long-life radical cation $ABTS^{+}$. This scavenging produces a decrease in the absorbance at 734 nm. ABTS (54.2 mg) was dissolved in phosphate buffer (pH 7,0.5 mM) and activated to $ABTS^{+}$ radical by addition of 1 g MnO2 with occasional stirring and time of activation 30 min (Pennycooke, Cox, & Stushnoff, 2005). Then the solution was centrifuged (5 min, 7000g), filtered (25 lm) and diluted with phosphate buffer. Sample addition was 10, 15 and 20µg/ml, time of reaction 20 min. Absorbance of the solution was measured at a wavelength of 734nm and quercetine (0.5mM) was used as standard [5].

E. Total phenolics

Total phenolic content of each *T.polium* extracts was determined with the Folin–Ciocalteau's reagent according to the published method (Shimadzu, Kyoto, Japan). Briefly, 0.1 ml aliquot of sample was mixed with 2.0 ml of 2% Na2CO3 and allowed to stand for 2 min at room temperature.0.1 ml of 50% Folin–Ciocalteau's phenol reagent was added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min in the dark. After incubation, absorbance of all the sample solutions was measured at 720 nm using spectrophotometer. Different volumes of quercetine(1mM) was used as standards[6].

F.Total flavonoids

The total flavonoid content of each *T.polium* extract was determined using a colorimetric method described by Kaijv, Sheng, and Chao (2006). To 0.25 ml of samples, 75 µl NaNO2 solution (5%, w/v), 0.15 ml AlCl3 solution (10%), and 0.5 ml NaOH solution (1 mol/l) were added. The final volume was adjusted to 2.5 ml with deionised water. The mixture solution was allowed to stand for 5 min and the absorption was measured at 507 nm against the same mixture, without the sample as a blank[7].

III. RESULTS AND DISCUSSION:

A. ABTS radical cation decolourisation assay showed differences in antioxidant potential between populations.

This method is based on measurements using ABTS.$^{+}$|ABTS redox couple in phosphate buffer solution, pH 7 [3]. ABTS radical cation decolourisation assay which is applicable for both lipophilic and hydrophilic antioxidants, showed various radical scavenging activity between *T.polium* populations. According to Figure 1 sample TPB had the highest ABTS radical scavenging activity after 20 minute[8].

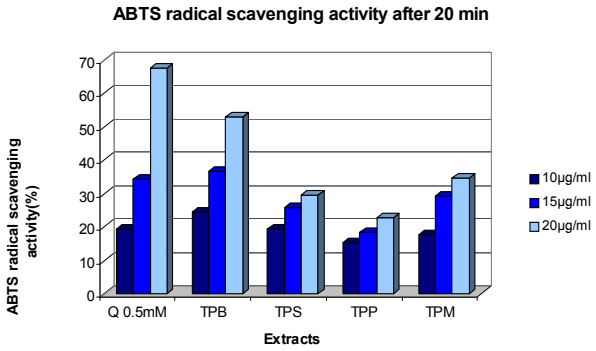

Figure 1. Radical cation scavenging activity (%) of the methanolic extracts were evaluated against radical cation ABTS+after 20 min.

B. The high antioxidant power is corrolated to the phenolic and flavonoid contents

Determination of total phenolic contents of different extracts of T. polium was done by using Folin– Ciocalteu colorimetric method. Phenolics including phenolic acids and flavonoids form a blue colour complex with phosphomolybdic–phosphotungstic acid reagent (Folin–Ciocalteu reagent) with maximum absorbance at 720 nm. Results are Expressed as micro mole quercetin equivalents per 100 micro gram of extract (Figure2) [1].

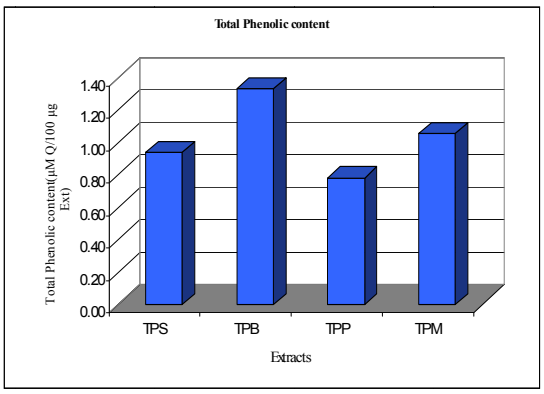

Figure 2. Total phenolic content (TPC) of aerial parts of extracts from T.polium. Results are Expressed as micro mole quercetin equivalents per 100 micro gram of extract

$AlCl_3$ colorimetric method was used for determination of total flavonoid contents of samples. In AlCl3 colorimetric method, aluminum chloride forms acid stable complex with the keto and/or the hydroxyl groups in the A or C ring of flavonoids, in addition it forms acid labile complexes with orthodihydroxyl groups of the A or B ring of flavonoids. The AlCl3 complexes of flavonoid compounds show strong

absorbance at 507 nm and flavonoids with more functional groups absorb stronger at 507 nm (Figure 3) [9].

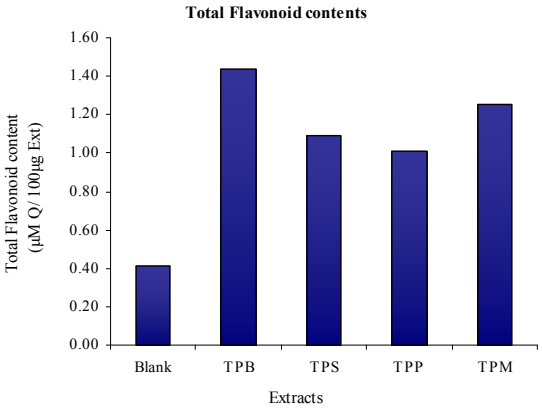

Figure 3. Total flavonoid content of each T.polium extract was determined using a colorimetric method. .We used the same mixture, without the sample as a blank. The absorption was measured at 507 nm.

Phenolic compounds represent a majority of the natural antioxidants presently identified. The most important classes of natural antioxidants include tocopherols, flavonoids and phenolic acids, which are common to all plant sources [10]. Results from Figures 4 and 5 showed that antioxidant power is correlated to the high phenolic and flavonoid contents in extracts.

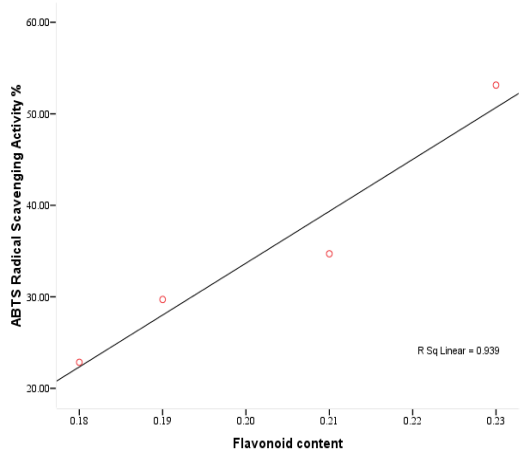

Figure 4. Correlation between *T.polium* organic extracts ABTS scavenging activity and their total Flavonoid contents.

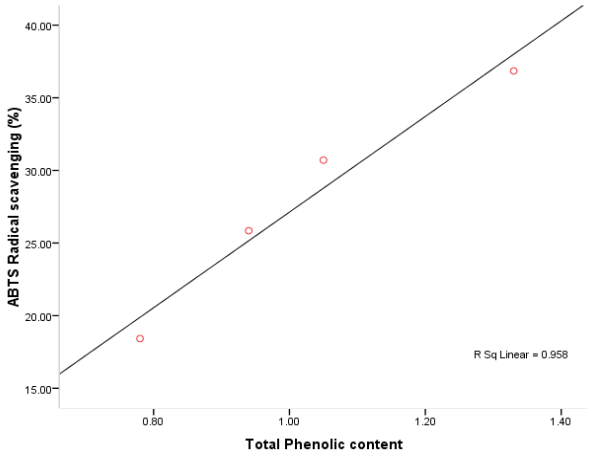

Figure 5. Correlation between T.polium organic extracts ABTS scavenging activity and their total phenolic contents.

IV. CONCLUSION:

These results indicate that the high ABTS radical scavenging ability of each *T.polium* extract can be predicted based on the determined total phenolic and flavonoid content. Sample TPB that showed the best antioxidant ability, is reach in phenolic and flavonoid contents.

These differences in antioxidant potencies and total phenolic and flavonoid contents among populations of *T.polium* may be explained by the variety of height and climate of growing regions.

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