

Total Phenolic Content and Antioxidant Activity of Two *Teucrium* Species from Syria

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Abstract: Germander (*Teucrium*, Lamiaceae) is a genus commonly used as a medicinal herb in the middle east. The aim of this study was to estimate total polyphenols and antioxidant activity of different extracts from two Syrian *Teucrium* species (*Teucrium polium* and *Teucrium creticum*). Total polyphenols content was measured using Folin-Ciocalteu assay and the antioxidant activity was evaluated *in vitro* by DPPH assay (free radical scavenging activity against 1, 1 – diphenyl-2-picrylhydrazyl). The total polyphenols content and antioxidant activity of *T. creticum* extracts were higher than *T. polium* ones. Phenols contents ranged from 16.388 to 105.672 mg GA E/ g dry weight extract. The extracts showed significant scavenging activity against DPPH, with IC50 values ranging between 0.195 and 0.710 mg/ml. The methanolic extracts show higher antioxidant activity from the two species. A good correlation between DPPH scavenging activities and contents of polyphenols was found. These results show that Syrian *Teucrium* species, especially *T. creticum* least Known among all, is a rich source of phenols and natural antioxidant compounds, that can be used as a natural food preservative.

Keywords: *Teucrium polium*, *Teucrium creticum*, Germander, Polyphenols, Antioxidant activity.

INTRODUCTION

During normal metabolism in aerobic cells, molecular oxygen is converted to water, then the stepwise transmit of electrons generates free reactive oxygen species (ROS) [1]. Massive generation of ROS and other radicals can damage important biomolecules such proteins, carbohydrates, fatty acids, and DNA, and may thus carry to oxidative intensity and to variety of degenerative processes and diseases, such as aging, neurologic disorders, inflammation, arteriosclerosis, cardio vascular disease, and certain cancers [2].

Plant phenolics were considered to have antioxidant activities due to their role as reducing agents, hydrogen donor antioxidant and singlet oxygen quenchers [3]. The genus *Teucrium* (Lamiaceae) contains 300 species spread all over the world, particularly in the Mediterranean basin [4]. In Syria there are more than 14 species. *T. polium* is the most Known and commonly used in folk medicine as an anti-gastrointestinal colic. It is named “Jaada” and “Quaisa”.

Meanwhile searching in the literature, numerous studies demonstrated anti-inflammatory [5], antibacterial [6], antiulcer [7], anti-nociceptive [8], antipyretic [9], hypolipidemic [10], hypoglycemic [11] effects of *T. polium*, as well as found to be used for treatment of hypertensive and intestinal troubles [12].

These therapeutic benefits of *T. polium* extracts are usually attributed to their ability to suppress oxidative process [13]. In this study, we aimed to highlight a new species of *Teucrium* (*T. creticum*) spread in Syria, which has not been studied yet, and compare their total poly phenols content and antioxidant activity with those of *T. polium*.

MATERIALS AND METHODS

Reagents and Standards

2,2-Diphenylpicryl hydroxyl radical (DPPH) and Folin-Ciocalteu were purchased from Sigma Aldrich, vitamin C and gallic acid from Prolabo, and sodium carbonate.

Solvents: chloroform, ethyl acetate and methanol were purchased from Surechem products LTD (SCP) with distillation range (95%) and all the other chemicals and solvents used were of analytical grade.

Plant Materials

Aerial parts of *T. polium* and *T. creticum* were collected from flowering plants in May 2018 from Homs and Lattakia (Syria) respectively. They were identified by Professor Imad Alkadi, Botanist at faculty of sciences, Damascus University, Syria.

Preparation of *Teucrium* Extracts

The plant material was dried at room temperature in a dark place, powdered, then extracted by progressive polarity solvents: chloroform, ethyl acetate, methanol

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and water (using 20 g of the powdered plant with 200 ml of each solvent). We obtained the chloroform, ethyl acetate, methanol extracts by soxhlet apparatus (continual extraction for 24 hours). Water extract by digestion and ultrasonication method. The resultant extracts were filtered and concentrated under vacuum using Rotary evaporator. The obtained dry extracts were stored in refrigerator at 4°C.

Statistical Analysis

All analysis was done triplicate, and values were then presented as average values along with their standard deviations. Data statistical analysis of scavenging activity assay and total phenol were performed by using one- way ANOVA followed by Tuckey-test. The level of significance was set at $p < 0.05$.

Determination of Total Phenolic Content

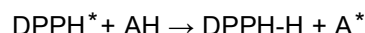
Total phenolic content of the extract was determined by the Folin-Ciocalteu method [14]. The 1 ml of known dilution of the extract, 9 ml of distilled water and 1 ml of Folin-Ciocalteu were mixed well for 5 minutes, then added to 10 ml of sodium carbonate solution 7%. The absorbance was read at 760 nm after 40 minutes of incubation at room temperature in the dark.

Gallic acid was used as a standard for the construction of a calibration curve, by preparing a series of standardized concentrations of gallic acid (5,10,15,20,25) mg/100ml ethanol 50% and apply the previously mentioned reaction Figure 1. Total phenolic values were calculated from the calibration curve $Y = 0.0433x + 0.0474$ with $R^2 = 0.995$, where x is the absorbance, y is the concentration of gallic acid. The

calibration method had been validated R^2 (correlation coefficient) = 0.995, SD (standard definition) = 0.67, RSD (relative standard definition) = 1.67%, LOQ (limit of quantification) = 13.2365 mg/l, LOD (limit of detection) = 4.3681 mg/l). Total phenol contents were expressed as milligrams of gallic acid equivalents per gram dry weight of extract (mg GA E/g DW).

DPPH Radical Scavenging Activity

The ability of the eight *Teucrium* extracts to scavenge DPPH free radicals was estimated using the method described by Tekao *et al.* [15] trusted with suitable modifications from Kumarasamy *et al.* [16] and vitamin C as a reference. The method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of the non-radical from DPPH-H by the reaction:



The solution of the plant extract was prepared in methanol to get the concentration of 1 mg/ml. Dilutions were made to achieve concentrations of 0.05, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH methanolic solution (80 µg/ml). After 30 minutes in darkness at room temperature, the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using equation:

$$\text{Inhibition (\%)} = 100 \times (\text{A of control} - \text{A of sample}) / \text{A of control}$$

Concentrations of extracts required to inhibit radical by 50% (IC50) values were estimated from the % inhibition versus concentration sigmoidal curve, using

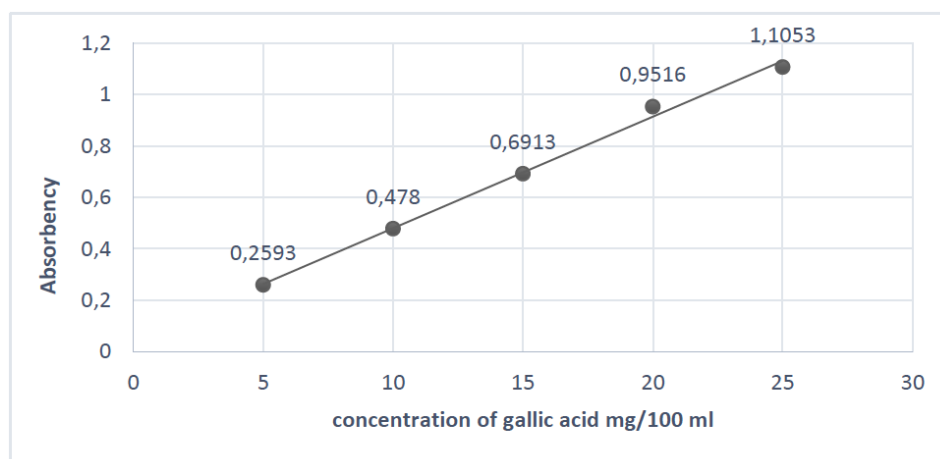


Figure 1: Standard curve of gallic acid.

Table 1: Extraction Yield of Different *T. polium* and *T. creticum* Extracts and TPC Expressed as mg GA E/DWE

TPC (mg GA E/DWE)	Extraction yield (%)	Type of extracts
32.304 ± 2.119	24.65 ± 1.04	Water E of <i>T. polium</i>
56.038 ± 3.351	17.75 ± 1.35	Water E of <i>T. creticum</i>
82.699 ± 4.204	14.95 ± 1.07	Methanol E of <i>T. polium</i>
105.672 ± 5.64	14.8 ± 1.53	Methanol E of <i>T. creticum</i>
35.122 ± 3.931	4.05 ± 1.72	Ethyl acetate E of <i>T. polium</i>
80.027 ± 3.930	4.6 ± 2.1	Ethyl acetate E of <i>T. creticum</i>
16.388 ± 1.388	6.35 ± 1.09	Chloroform E of <i>T. polium</i>
24.344 ± 1.353	4.65 ± 1.85	Chloroform E of <i>T. creticum</i>

Each value expressed as mean of three replicate ± standard deviation, E = extract. (%) = mg extract/100 g dry weight plant. TPC = Total Phenolic Content. mg GA E/DWE = milligrams of gallic acid equivalence in dry weight extract.

anon-linear regression analysis. The data was presented as mean values ± standard deviation (N = 3).

RESULTS AND DISCUSSION

Extraction Yield

There are a lot of factors that effect the extraction yield like the method of extraction, repetition and time extraction, the solvent used, sample size, presence of overlapping materials as well as chemical nature of the phytochemicals [17]. In this work extraction yield as illustrated in Table 1 ranged from 4.05% to 24.65% in *T. polium* and from 4.6% to 17.75% in *T. creticum*, the highest extraction yield is noticed in water and methanol extracts. These results may be due to the

higher solubility of proteins, carbohydrates, pigments and other secondary metabolites in polar solvents than nonpolar ones.

Total Phenolic Contents

The distribution of phenolic content in *T. polium* and *T. creticum* is presented in Figure 2 and Table 1. The content of total phenolic compounds in extracts, which is expressed as milligrams of gallic acid equivalents per gram of dry weight extract (mg GA E/g DWE), ranged between 16.388 ± 1.388 to 105.672 ± 5.64 mg GA E / g DWE. The results in Figure 2 indicates a significant difference between concentrations of phenol in different solvent extracts. Phenolic contents were the highest in methanol extracts from both plants. Water and ethyl acetate extract also contained high level of phenols, while contents of phenols in chloroform extracts was

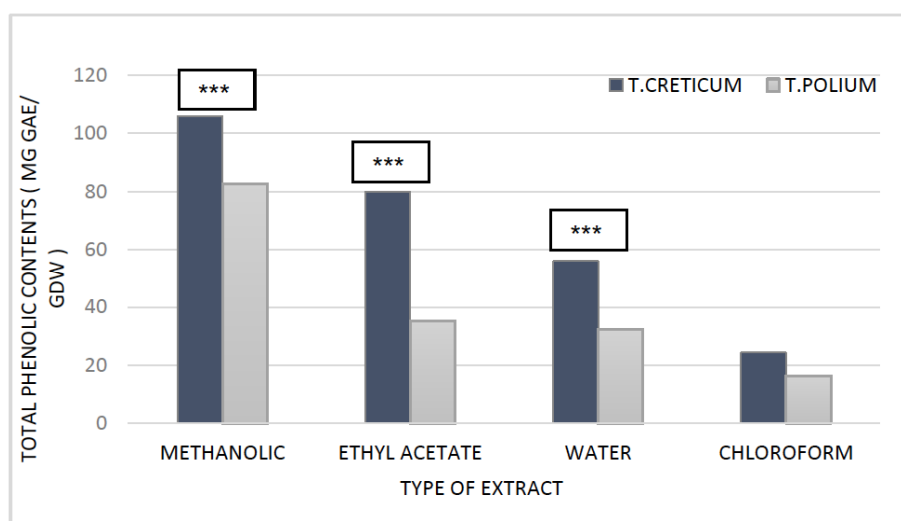


Figure 2: Total phenolic compounds of extracts from *T. polium* and *T. creticum*.

Results were expressed as mg GAE/g dry weight. Each value represents means ± SD of three experiments (**p < 0.05).

Table 2: IC50 in DPPH Radical Scavenging Activity (mg/ml) of *T. creticum* and *T. polium* Extracts Compared to that of Vitamin C (IC50 = 0.017 ± 0.0001 mg/ml)

Chloroform	Ethyl acetate	Water	Methanolic	Type of extract
0.52 ± 0.005	0.33 ± 0.006	0.20 ± 0.06	0.19 ± 0.007	<i>Teucrium creticum</i>
0.71 ± 0.028	0.36 ± 0.009	0.30 ± 0.068	0.26 ± 0.004	<i>Teucrium polium</i>

Each value expressed as mean of three replicate ± standard deviation. The extracts of the same solvent and vitamin C are significantly different ($p < 0.05$).

the lower. In all extracts, the Content of phenols was higher in *T. creticum* than *T. polium*. The highest total phenolic concentration is noticed with the methanolic extract, this result was in agreement with many previous studies (Y *et al.* 2019 [18], Stankovic *et al.* 2012 [19]).

Previous study reported that the variability in the total phenolic contents in different extracts could be the result of many reasons like: varying solubility of the phenolic compounds, method of extraction, the region of the plant, the variation in solubility may be driven by the solvent polarity [20].

Antioxidant Activity

The antioxidant activity of *Teucrium* extracts was investigated by the DPPH radical scavenging assay. Results in Table 2 show the IC50 values in DPPH radical scavenging activity assay of *T. polium* and *T. creticum* extracts, the IC50 values of all tested samples ranged from 0.2 to 0.854 mg/ml. The results indicate that *T. creticum* has higher activity than *T. polium* in comparison to the same solvent extract which agree with the high content of poly phenols in *T. creticum* extracts. All extracts show a significant inhibition of the DPPH radical as follows:

methanol > water > ethyl acetate > chloroform.

The higher activity noticed with polar solvent extracts (methanol and water) may be attributed to the type of polyphenols existed in methanol and water extracts in glycoside form, that may have a larger number of electron-donating hydroxyl groups relative to polyphenols in ethyl acetate and chloroform extracts. Since an antioxidant agent is considered to be active against free radicals if IC50 is less than 5 mg/ml [21] therefore all our extracts can serve as a strong natural antioxidant.

Correlation of Antioxidant Activity with Phenolic Content

We found that there was a significant correlation between the total phenolic contents with free radical

scavenging activity in methanol, ethylacetate and chloroform extracts ($r^2 = 0.7$) for *T. polium* and ($r^2 = 0.9$) for *T. creticum*. The free radical scavenging activity against DPPH in our work could be attributed to the phenolics content. According to the previous researches, phenol compounds are recognized as anti-cancer, anti-inflammatory, anti-bacterial agents because of their free radical scavenging properties [22]. Likewise, there are many benefits of *Teucrium* species due to their high phenolic contents and antioxidant activities.

CONCLUSION

Depending on the results we obtained from this study, *T. polium* and *T. creticum* extracts have a remarkable antioxidant effect due to their important content of polyphenols. Therefore, they could serve as a strong protective agent against free radical associated oxidative damage. To the best of our work, this is the first report comparing the antioxidant activity of two Syrian *Teucrium* species. From the research, we found that *T. polium* spread in Syria have a lower polyphenol content than the species spread in Serbia [19] and Morocco [18]. Another thing observed was that the *T. creticum* in Syria that was studied for the first time had a higher number of polyphenols and a stronger antioxidant activity than *T. polium*.

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CONFLICT OF INTEREST

There is no conflict of interest.

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