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Chemical Composition and *in vitro* Evaluation of Total Phenolic, Flavonoid, and Antioxidant Properties of Essential Oil and Solvent Extract from the Aerial Parts of *Teucrium polium* Grown in Tunisia

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Abstract Total phenolic and flavonoid contents and antioxidant properties of extracts and essential oils of the aerial parts of *Teucrium polium* growing in Tunisia were evaluated. Total phenolic and flavonoid contents of extracts ranged from 48.88±0.01 to 400.00±0.01 mg of GAE/g and from 2.75±0.01 to 38.85±0.04 mg of QE/g, respectively. GC/MS analysis of essential oils led to identification of 71 compounds representing 89.66% of the total composition. Antioxidant activities were assessed using the DPPH· (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, β-carotene bleaching, and reducing power. The best overall antioxidant activity was recorded for acetone extracts, with a strong positive correlation between the total phenolic content and the IC_{50} value of DPPH (R^2=0.9999), attributed to the polyphenolic content.

Keywords: *Teucrium polium*, extract, essential oil, total phenolic and flavonoid content, antioxidant activity

Introduction

Essential oils and extracts from plants, herbs, and spices have long been used for culinary and medicinal purposes throughout the world. Even with recent advances in medical and pharmaceutical industries, natural products have not lost their place in modern therapy. In fact, growing concerns over the escalation food-borne diseases, multiple drug resistances, and side effects associated with synthetic substances have renewed interest in use of plant-derived agents for therapeutic and medicinal purposes.

Several studies have reported isolation and/or synthesis of valuable compounds from plants throughout the world (1). In this context, the flora of Tunisia is known to have a rich source of medicinal and aromatic plant species that produce a wide range of attractive natural substances, essential oils, and organic compounds (2,3). Of particular interest, *Teucrium polium,* commonly known as felty germander, is a member of a large genus of perennial plants in the Lamiaceae family that comprises more than 200 species of herbs, shrubs, and subshrubs (4). Plant species in this genus are 20-50 cm high shrubs and woody herbs widely distributed in southwestern Asia, Europe, and North Africa (5) and have been used for thousands of years in traditional medicine (6,7) for diuretic, diaphoretic, tonic, antipyretic, antispasmodic, and cholagogue purposes (8-12). Species of the *Polium* genus are rich in monoterpenes, sesquiterpenes, saponins, sterols polyphenolic compounds, flavonoids, alkaloids, and essential oils (13-18) and have also been reported to exhibit a wide range of beneficial activities, including antioxidant, anti-inflammatory, antinociceptive, antibacterial, anti-hypertensive, anticancer, and hypoglycemic (6,7,19-22).

Despite a large amount of information regarding several *T. polium* species from around the world, research concerning crude solvent extracts and essential oils of Tunisian *T. polium* is limited with no available reports on antioxidant activity. Accordingly, this study investigated and evaluated the chemical composition, total phenolic and flavonoid contents, and *in vitro* antioxidant activities of dichloromethane, acetone, ethanol, hydroalcoholic, and aqueous extracts and essential oils from the aerial parts of *T. polium* growing in Tunisia using 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing

Antioxidant Power (FRAP), and β-carotene bleaching assays.

Materials and Methods

Reagents DPPH, β-carotene, ascorbic acid (AA), Folin-Ciocalteu reagent, sodium carbonate, linoleic acid, ferric chloride ($FeCl_3$), trichloroacetic acid, butylated hydroxytoluene (BHT), potassium ferrocyanide ($K_3Fe(CN)_6$), ferrous sulfate ($FeSO_4$), potassium persulfate ($K_2S_2O_8$), Tween 60, sodium chloride, and solvents used for extraction and partition were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Spectrophotometric measurements were performed using a double-beam UV-VIS spectrophotometer.

Plant materials Aerial parts of *T. polium* were collected during the flowering stage in June of 2013 from Oued Sarrath (altitude, 602 m; latitude, 35°46; longitude, 8°37) in the region of Kef in southwest Tunisia. Plants were identified based on comparison with the flora of Tunisia (23) and voucher specimens were deposited in the herbarium of the biology department at the Faculty of Science of Sfax.

Extraction of essential oils Dried powder of the aerial parts of the *T. polium* (700g) was subjected to hydrodistillation (6) for 4 h using a clevenger type apparatus. Resulting essential oils were dried over anhydrous sodium sulfate, and stored in sealed vials in the dark, at 4°C for subsequent analysis.

Preparation of extracts Aerial parts of *T. polium* (100 g) were dried at 50-60°C for two days powdered in a mechanical grinder, and then subjected to extraction twice using maceration with dichloromethane (DC), acetone, ethanol, hydroalcohol (1:2, v/v) and water. Extract solutions were filtered with filter paper and then collected and concentrated using a rotary evaporator at 35-55°C. Extracts thus obtained were kept in the dark at 4°C until further use. The yield of evaporated dried extracts was calculated based on dry weight as:

$$\text{Yield (\%)} = (W1\times100)/W2$$

where $W1$ is the extract weight after evaporation of the solvent and $W2$ is the dry weight of a plant material.

Chemical analysis

GC–MS: GC-MS analysis was performed on an Agilent 6890 series gas chromatograph interfaced with an Agilent 19091S-433 (Agilent Technologies, Santa Clara, CA, USA). The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 μm) HP-5MS (5% phenyl) methyl siloxane capillary column. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The constituents of essential oils were identified based on comparison of Kovats retention indices (RI) (24) using a homologous series of *n*-alkanes (C9-C22) under the same GC-MS conditions.

Total phenolic contents: The total phenolic contents of extracts were determined using the Folin-Ciocalteu method (25,26). Results were expressed as gallic acid equivalents per g of dry weight (mg of GAE/g of dry extract weight).

Total flavonoid contents: The flavonoid contents of extracts were determined following the Bahorun and Shahidi (27) method. A quercetin calibration curve was prepared by mixing 1 mL of sample (0-100 μg/mL) and a quercetin methanol solution with 1 mL of an aluminium trichloride solution (2% in methanol). After 10 min, the absorbance was measured using a spectrophotometer at 415 nm. Results were expressed as quercetin equivalents (QE/g of dry sample weight).

Determination of antioxidant activity

DPPH· radical scavenging assay: The antioxidant activities of extracts and essential oils were analyzed using the method of Sanchez-Moreno *et al.* (28). Reaction mixtures containing 0.5 mL of a sample concentration of 0.025 to 0.1 mg/mL were each mixed with 1 mL of a DPPH solution (2 mg dissolved in 50 mL of methanol). Samples were kept in the dark for 30 min at room temperature (298 K) and the absorbance was measured using a spectrophotometer against a blank at 517 nm. The antioxidant activities of samples for scavenging DPPH radicals was calculated as:

$$I\ (\%)=[(A_0-A_1)/A_0)]\times100$$

where A_0 is the absorbance of a blank and A_1 is the absorbance of a sample. BHT and ascorbic acid were used as positive controls.

Reducing power assay (FRAP): The ferric reducing power was determined using a slightly modified version of the method described by Oyaizu *et al.* (29). In brief, 250 μL of a sample at a concentration of 0.0625 to 1 mg/mL was mixed with a phosphate buffer (500 μL, 0.2 M, pH 6.6) and potassium ferricyanide (500 μL, 1%). Mixtures were then incubated water bath at 50°C for 20 min. Then, 500 μL of trichloroacetic acid (10%) was added to each sample and mixtures were centrifuged at 1,006×*g* for 10 min. After that, 750 μL of the upper layer was mixed with 750 μL of distilled water, then 50 μL of ferric chloride (0.1%) was added and the absorbance was measured at 700 nm against a control consisting of all reagents without a test sample.

β-Carotene bleaching: Antioxidant assays were performed based on the method of Koleva *et al.* (30) with slight modification. In brief, 0.5 mg of β-carotene was dissolved in 1 mL of chloroform and mixed with 25 μL of linoleic acid and 200 μL of Tween 60. The absorbance was measured using a spectrophotometer against a blank at 470 nm. The antioxidant activities of samples were compared with activities of BHT and a blank. The antioxidant activities of sample fractions were evaluated as β-carotene bleaching inhibition (%)=[1–(OD_0–OD_{120})/(OD'_0–OD'_{120})]x100, where OD'_0 and OD'_{120} are absorbance values of a control at 0 and 120 min, respectively, and OD_{120} is the absorbance value of a sample at 120 min.

Statistical analysis All numeric values represent mean values of 3

measurements±standard deviation (SD). Correlations between results were determined using an analysis of variance (ANOVA) and quantified based on the correlation coefficient (R^2). Differences were considered significant at $p<0.05$.

Results and Discussion

Extraction yield and total phenolic and flavonoid contents Extraction yields obtained from the aerial parts of *T. polium* are shown in Table 1. Recovery percentage values of extractable compounds ranged from 2.34 to 9.10% (w/w). Aqueous extracts gave the maximum yield of 9.10%, followed by ethanol (7.99%) and hydroalcoholic (7.92%) extracts. Variations in extract yields were probably related to the polarity of extracted components and solvents used (31). Water as a universal solvent is generally used in traditional settings for preparation of plant decoctions for health remedies. High extraction yields previously obtained using water indicated that pigments, enzymes, and bioactive components were soluble in water (32).

The biological activity of polyphenolic compounds may be related to chelation of metals, inhibition of lipoxygenase, and scavenging of free radicals (33). Amounts of total phenols and flavonoids were determined using Folin-Ciocalteu and quercetin reagents, respectively. Gallic acid and quercetin were used as standard compounds and results were expressed as mg/g of gallic acid and mg/g of quercetin equivalents, respectively. The total phenolic content varied from 48.88±0.01 to 400.00±0.01 mg of GAE/g, with the highest phenolic content recorded for acetone extracts (Table 1). The total flavonoid content, on the other hand, ranged between 2.75±0.01 and 38.85±0.04 mg of QE/g, with the acetone extract exhibiting the maximum flavonoid content. Significant ($p<0.05$) differences existed between amounts of total phenols and flavonoids.

High total antioxidant activities were probably related to phenolic compounds present in extracts. Although the mechanism responsible for the presence of phenolic compounds has not yet been fully elucidated, involvement of interactions between acetone and polyphenolic compounds is probable. In fact, the presence of acetone as a carbonyl gives a polar character to the molecule and the presence of phenolic and flavonoid compounds with hydroxyl groups also confers a high degree of polarity on these molecules. The interactive abilities of acetone and polyphenolic compounds are probably related to chemical compositions and structures. Content variability in extracts depended on the extraction solvent (34) and probably resulted from different degrees of solubility of different compounds which, in turn, was probably related to the degree of solvent polarity. Phenolic compositions of extracts can also be affected by the plant variety and ecological and storage conditions.

Chemical composition of essential oils Essential oils hydrodistilled from the aerial parts of *T. polium* were colorless with an aromatic odor and were obtained at a yield of approximately 0.7%. Percentage compositions, retention indices, and identification methods are listed in Table 2 where components are arranged in order of elution from an HP-5MS column. GC/MS analysis of essential oils led to identification of 71 compounds representing 89.66% of the total composition. The major components in essential oils were α-pinene (17.04%), β-pinene (12.68%), and limonene (6.65%), followed by β-myrcene (6.07%) and germacrene D (5.89%). Essential oils also contained a large proportion of terpenoid hydrocarbons (68.87%),

Table 1. Extract yields and total phenolic and flavonoid contents of *T. polium* extracts

Extracts	Yield (%)	Phenolics (mg of GAE/g of extract)	Flavonoids (mg of QE/g of extract)
Dichloromethane	3.71±0.30[1)]	48.88±0.01	33.56±0.04
Acetone	2.34±0.01	400.00±0.01	38.85±0.04
Ethanol	7.99±0.01	80.00±0.01	11.95±0.04
Hydroalcoholic	7.92±0.02	183.33±0.01	11.37±0.03
Aqueous	9.12±0.01	277.77±0.05	2.75±0.01

[1)]Values are expressed as a mean±SD of 3 independent determinations.

Table 2. Chemical composition of essential oils isolated from the aerial parts of *Teucrium polium* from Tunisia

No.	Compounds	Area (%)	RI[2)]	Identification
1	α-Thujene[1)]	2.87	931	RI, MS
2	α-Pinene	17.04	940	RI, MS
3	Camphene	0.37	949	RI, MS
4	Verbenene	0.27	955	RI, MS
5	β-Pinene	12.68	979	RI, MS
6	Octen-3-ol	0.45	983	RI, MS
7	β-Myrcene	6.07	994	RI, MS
8	α-Terpinene	0.09	1016	RI, MS
9	*p*-Cymene	1.06	1025	RI, MS
10	Limonene	6.65	1031	RI, MS
11	(*Z*)-β-Ocimene	0.20	1039	RI, MS
12	(*E*)-β-Ocimene	0.69	1049	RI, MS
13	γ-Terpinene	0.17	1059	RI, MS
14	Linalool oxide	0.13	1073	RI, MS
15	α-Terpinolene	0.28	1089	RI, MS
16	Fencholenic aldehyde	0.17	1090	RI, MS
17	Linalool L	1.30	1103	RI, MS
18	β-Thujone	0.24	1117	RI, MS
19	α-Campholene aldehyde	0.74	1127	RI, MS
20	β-Pinone	0.29	1138	RI, MS
21	Trans-Pinocarveol	1.52	1141	RI, MS
22	*cis*-Verbenol	0.57	1143	RI, MS
23	Verbenol	2.49	1149	RI, MS
24	Pinocarvone	0.31	1163	RI, MS
25	Borneol	0.30	1168	RI, MS
26	*p*-Mentha-1,5-dien-8-ol	0.20	1170	RI, MS
27	4-Terpineol	0.84	1179	RI, MS
28	*p*-Cymene-8-ol	0.38	1190	RI, MS
29	α-Terpineol	0.70	1194	RI, MS
30	Myrtenal	1.22	1197	RI, MS

Table 2. Continued

No.	Compounds	Area (%)	RI	Identification
31	Myrtenol	0.90	1199	RI, MS
32	Verbenone	0.88	1211	RI, MS
33	Trans-Carveol	0.48	1222	RI, MS
34	Benzaldehyde,4-(1-methylethyl)	0.09	1242	RI, MS
35	l-Carvone	0.32	1246	RI, MS
36	Borneol, acetate	0.43	1286	RI, MS
37	Sabinyl acetate	0.16	1325	RI, MS
38	1,5,5-Trimethyl-6-methylenecyclohexene	0.05	1336	RI, MS
39	Carene	0.24	1349	RI, MS
40	α-Copaene	0.21	1375	RI, MS
41	β-Bourbonene	0.63	1385	RI, MS
42	β-Cubebene	0.42	1389	RI, MS
43	β-Elemene	0.20	1391	RI, MS
44	Trans-Caryophyllene	0.98	1419	RI, MS
45	α-Bergamotene	0.13	1434	RI, MS
46	α-Guaien	0.51	1438	RI, MS
47	α-Humulene	0.59	1453	RI, MS
48	Aromadendrene	0.25	1460	RI, MS
49	Epizonaren	0.22	1474	RI, MS
50	α-Amorphen	0.24	1478	RI, MS
51	Germacrene D	5.89	1484	RI, MS
52	β-Selinene	0.17	1487	RI, MS
53	Bicyclogermacren	2.63	1497	RI, MS
54	α-Muurolene	0.27	1499	RI, MS
55	δ-Guaiene	0.55	1505	RI, MS
56	α-Gurjunene	0.41	1508	RI, MS
57	α-Cadinadiene	0.32	1514	RI, MS
58	δ-Cadinene	3.86	1524	RI, MS
59	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	0.11	1532	RI, MS
60	Elemol	0.19	1550	RI, MS
61	Germacrene B	0.28	1557	RI, MS
62	Bisabolene oxide	0.18	1559	RI, MS
63	Spathulenol	2.36	1580	RI, MS
64	Caryophyllene oxide	0.58	1584	RI, MS
65	Nor-Copaanone	0.08	1624	RI, MS
66	γ-Muurolene	0.32	1648	RI, MS
67	β-Eudesmol	0.72	1653	RI, MS
68	α-Cadinol	1.70	1657	RI, MS
69	α-Selinene	0.09	1768	RI, MS
70	β-Panasinsene	0.67	1786	RI, MS
71	Phytol	0.06	2010	RI, MS
	Total	89.66		
	Monoterpene hydrocarbons	48.73		
	Oxygenated monoterpenes	14.50		
	Sesquiterpene hydrocarbons	20.14		
	Oxygenated sesquiterpenes	5.54		
	Others	0.75		

[1)]Components and percentages are listed in order of elution on a polar column (HP-5MS)

[2)]RI: Retention Indices

represented by monoterpene (48.73%) and sesquiterpene (20.14%) hydrocarbons and lower proportions of oxygenated terpenoids (20.04%) (represented by oxygenated monoterpenes, 14.50%) and sesquiterpenes (5.54%). All other components were present at less than 1%.

Extracts and chemical compounds from different parts of *T. polium* exhibited a broad range of biological activities, including hepato-protective, antimutagenic, anticancer, hypolipidemic, anti-inflammatory, antinociceptive, antispasmodic, hypoglycemic, hypotensive, antiulcer, and antimicrobial properties (32). Different chemical compositions have been reported for essential oils from *T. polium* (35). Results of this study were similar to a previous report (33) in which variations in volatile compounds among Tunisian *T. polium* populations and relationships with bioclimate and ploidy level were investigated. Identification of 38 compounds showed myrcene (15.3%), germacrene D (9.0%), β-pinene (6.6%), β-pinene (5.8%), and β-cadinol (5.1%) to be the primary compounds, with significant variations among populations. The major components of *T. polium* essential oils were sesquiterpenes consisting mostly of caryophyllene and germacrene D (32).

Menichini *et al.* (12) reported that *T. polium* essential oils contained 70 components with different distributions, including carvacrol (10.1%), caryophyllene (9.8%), torreyol (7.6%), and caryophyllene oxide (5.0%), with a prevalence of sesquiterpene hydrocarbons (32.7%) over oxygen-containing sesquiterpenes (23.1%). Moghtader (36) reported essential oil compositions from *T. polium* in Iran and identified 28 compounds representing 99.7% of the total oils with 0.75% oil yields. More recently, Belmekki *et al.* (37) identified 27 components from Algerian *T. polium* essential oils with the major compounds of germacrene D (25.81%), bicyclogermacrene (13%), β-pinene (11.69%), and carvacrol (8.93%). Differences in chemical compositions of oils were presumably related to different ecologic parameters of plant age, developmental stage, and geographical origin, and to climatic and genotypical factors. Furthermore, the lower antioxidant activities recorded for essential oils, compared to the AA standard, can be attributed to the presence of the monoterpenes β-pinene and β-pinene (38).

Antioxidant activity Different *T. polium* essential oils and extracts were subjected to screening for antioxidant activity using the 3 spectrophotometric methods of DPPH, β-carotene, and FRAP.

DPPH free radical-scavenging activity: The effect of antioxidants on DPPH radical scavenging is generally attributed to a hydrogen-donation ability (39,40). The reaction between an antioxidant and DPPH depends heavily on the structural conformation of the antioxidant, and some compounds are known to react quickly with DPPH, leading to reduction of DPPH molecules equal to the number of hydroxyl groups (41). Plant fraction and essential oil samples were free radical scavengers and the scavenging effect on the DPPH radical varied in a dose-dependent manner (Fig. 1A). The scavenging activities of extracts based on inhibition percentages varied with the extraction solvent used in the order of ethanol>acetone> hydro-alcoholic>aqueous>DC. The percentage recorded for essential oils was 62%. Inhibitory concentrations required for each fraction to

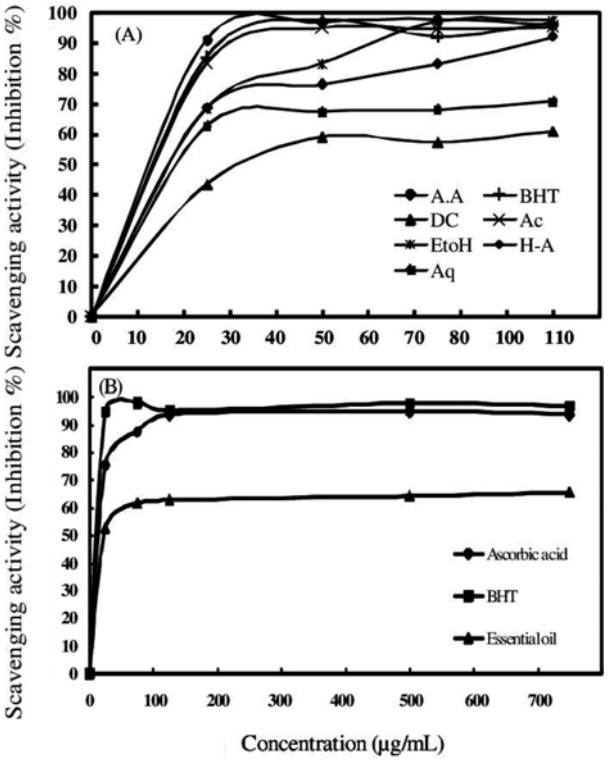

Fig. 1. Antioxidant activities of the aerial parts of *Teucrium polium.* (A) Scavenging activities of *Teucrium polium* extracts against DPPH radicals, (B) Scavenging activities of essential oils of *Teucrium polium* against DPPH radicals. A.A, Ascorbic acid; BHT, butylated hydroxytoluene; DC, dichloromethane; Ac, Acetone; EtOH, Ethanol; H-A; Hydroalcoholic; Aq, Aqueou

scavenge 50% of the DPPH radical (IC_{50} value) were compared with AA and BHT as reference standards (Table 3). Higher DPPH radical-scavenging activities were associated with lower IC_{50} values. Acetone extracts exhibited the greatest antioxidant activities with an IC_{50} value of 13±0.02 μg/mL, which was close to reference standard values (13±0.01 μg/mL) followed by hydroalcoolic, aqueous, and ethanol extracts. The lowest DPPH radical-scavenging activity was observed for DC extracts (32±0.03 μg/mL).

T. polium essential oils showed an IC_{50} value of 20 μg/mL, which was lower than for AA and BHT (Fig. 1B). The final acetone extract solution after completion of the reaction always showed some yellowish color, suggesting that the acetone extract had a high DPPH scavenging activity. A strong positive correlation was identified (R^2= 0.9999) between the radical scavenging activity evaluated based on the DPPH assay and the phenolic content of *T. polium*, suggesting that a high phenolic concentration of the acetone extract was responsible for the antioxidant activity, in agreement with previous reports attributing the DPPH scavenging ability of essential oils to the presence of α-pinene, β-pinene, *p*-cymene, and borneol, all components with antioxidant activities (38).

β-Carotene-linoleic acid assay: The potential of plant extracts and essential oils to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation was assessed based on a β-carotene-linoleic acid assay**.** The β-carotene bleaching method is based on loss of the yellow color of β-carotene, which is monitored spectrophotometrically, due to a reaction with radicals that are formed by linoleic acid oxidation. Compared with BHT, aqueous extracts exhibited the greatest degree of inhibition (60%), followed by hydroalcoholic (49%), ethanol (46 %), acetone (42%), and dichloromethane (40%) extracts (Fig. 2A and Table 3). Aqueous extracts showed the best inhibition potential against oxidation of linoleic acid with an IC_{50} value of 781 μg/mL, which was significantly (p<0.05) lower (9.8875) than the value for the BHT reference standard (IC_{50}=79 μg/mL), perhaps attributable to a high phenolic content (277.77 mg of GAE/g of dry weight of extract). The hydrodistilled oil of *T. polium* was less effective with an IC_{50} value=150 μg/mL, which was approximately 2x lower than the value for the BHT standard (Fig. 2B).

Ferric ion reducing power: The presence of reducers causes transformation of the Fe^{3+} ferricyanide complex into Fe^{2+} due to donation of an electron. Production of Fe^{2+} can be monitored based on measurement of Perl's Prussian blue formation at 700 nm. Dose-response curves for the reducing power of *T. polium* extracts and essential oils, compared with AA as a positive control, are shown in Fig. 3A and 3B. At a concentration of 1,000 g/mL, acetone extracts showed the greatest reducing power with an absorbance value of A_{700}=0.92, whereas dichloromethane extracts exhibited the lowest

Table 3. Antioxidant activities of essential oils and solvent extracts from the aerial parts of *Teucrium polium*

	DPPH		β-Carotene		FRAP
Extract	IC_{50} (μg/mL)[1)]	Maximum inhibition (%)	IC_{50} (μg/mL)	Maximum inhibition (%)	EC_{50} (μg/mL)
Essential oil	20±0.01	66.01	150±0.01	80	(-)
Dichloromethane	32±0.03	60.65	(-)[2)]	40	38±0.03
Acetone	13±0.02	95.06	(-)	42	18±0.02
Ethanol	19±0.02	97.13	(-)	46	20±0.02
Hydroalcoholic	17±0.04	91.84	(-)	49	27±0.04
Aqueous	19±0.02	70.49	781±0.03	60	(-)
Ascorbic acid	12±0.01	97.42	(-)	(-)	25±0.01
BHT[3)]	13±0.01	94.38	79±0.01	100	(-)

[1)]Values corresponding to the concentration of extracts/essential oils required for inhibition of 50% of radicals in the reaction mixture.
[2)](-): Not detected.
[3)]Butylated hydroxytoluene.

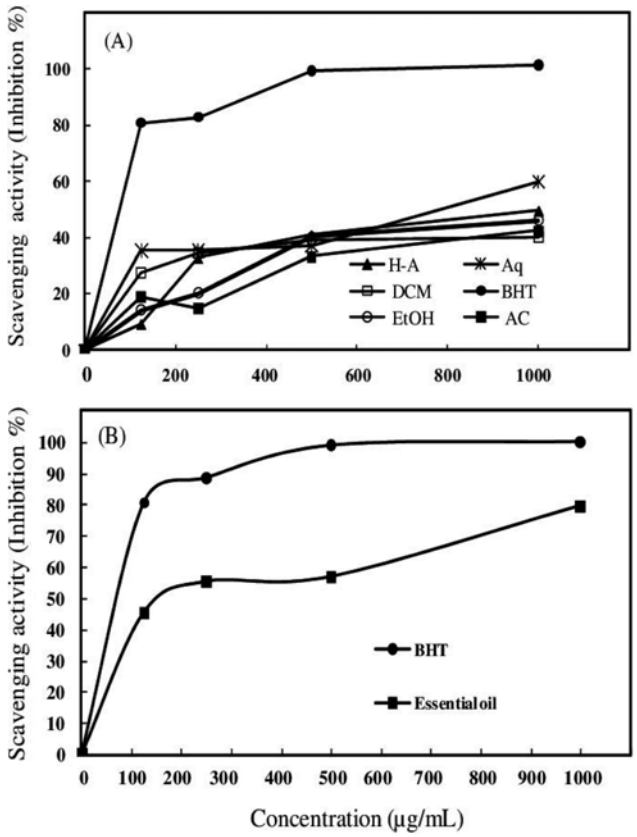

Fig. 2. Antioxidant activities of the aerial parts of *Teucrium polium.* (A) β-Carotene-linoleic acid assay of *Teucrium polium* extracts, (B) β-Carotene-linoleic acid assay of *Teucrium polium* essential oils. A.A, Ascorbic acid; BHT, butylated hydroxytoluene; DC, dichloromethane; Ac, Acetone; EtOH, Ethanol; H-A; Hydroalcoholic; Aq, Aqueou

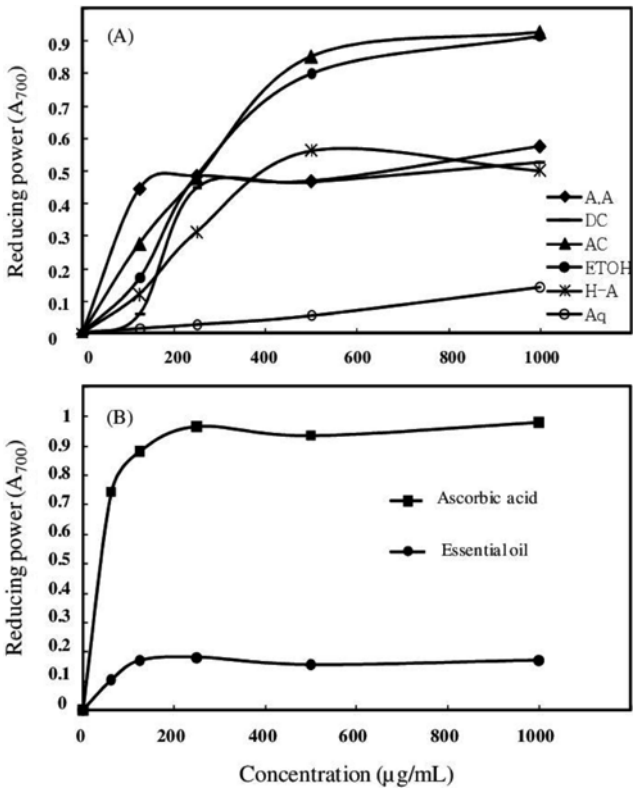

Fig. 3. Antioxidant activities of the aerial parts of *Teucrium polium.* (*A*) Chelating activites of *Teucrium polium* extracts against Fe^{2+}, (B) Chelating activities of *Teucrium polium* essential oils against Fe^{2+}. A.A, Ascorbic acid; BHT, butylated hydroxytoluene; DC, dichloromethane; Ac, Acetone; EtOH, Ethanol; H-A; Hydroalcoholic; Aq, Aqueou

activity of A_{700}=0.15. The highest ferric reducing power of EC_{50}=18 μg/mL was recorded for acetone extracts, followed by ethanol (EC_{50} = 20 μg/mL) and hydroalcoholic (EC_{50}=27 μg/mL) extracts (Table 3). The lowest ferric reducing power of EC_{50}=38 μg/mL was observed for dichloromethane extracts. *T. polium* essential oils exhibited a low ferric reducing power capacity (Table 3). Reducing power has generally been correlated with the amount and composition of plant extracts and essential oils, which can be affected by variety, climate, and storage (38).

Overall, antioxidant properties of plant extracts are considered to stem from synergistic effects. Radical scavenging values in plant species are dependent on area of origin and extraction solvent polarity (42). Previous pharmacological studies showed that terpenic and polyphenolic compounds were responsible for the pharmacological activities of different *Teucrium* species (43).

In conclusion, *T. polium* extracts exhibited powerful antioxidant activities with acetone as the most efficient solvent for phenolic and flavonoid extraction. A good correlation was also observed between acetone extracts and the presence of high phenolic and flavonoid contents, which were probably the major contributors to antioxidant activities. *T. polium* extracts and essential oils are promising candidates for development of effective bioactive compounds and antioxidant agents for applications in medical, therapeutic, and pharmacological products and formulations.

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References

1. Le Floc'h E. Contribution to ethnobotanical study of the flora of Tunisia. Program flora and vegetation in Tunisia. Tunisian Scientific Publications, Official Printing Office of the Republic of Tunisia, Tunis, Tunisia. pp. 36-37 (1983)
2. Brusotti G, Cesari I, Frassà G, Grisoli P, Dacarro C, Caccialanza G. Antimicrobial properties of stem bark extracts from *Phyllanthus muellerianus* (Kuntze) Excell. J. Ethnopharmacol. 135: 797-800 (2011)
3. Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Efferth T. Gems from traditional north-African medicine: Medicinal and aromatic plants from Sudan. Nat. Prod. Bioprospect. 2: 92-103 (2012)

4. Wielgorskaya T. Dictionary of Generic Names of Seed Plants. Columbia University Press, New York, NY, USA. pp. 304-309 (1995)
5. Djabou N, Allali H, Battesti M J, Tabti B, Costa J, Muselli A, Varesi L. Chemical and genetic differentiation of two Mediterranean subspecies of *Teucrium scorodonia* L. Phytochemistry 74: 123-132 (2012)
6. Abdollahi M, Karimpour H, Monsef-Esfehani HR. Antinociceptive effects of *Teucrium polium L*. total extract and essential oil in mouse writhing test. Pharmacog. Res. 48: 31-35 (2003)
7. Tariq M, Ageel AM, Al-Yahya MA, Mossa JS, Al-Said MS. Antiinflammatory activity of *Teucrium polium*. Int. J. Tissue React. 11: 185-188 (1989)
8. Al-Khalil S. A survey of plants used in Jordanian traditional medicine. Int. J. Pharmacogn. 33: 317-323 (1995)
9. Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J. Ethnopharmacol. 83: 251-265 (2002)
10. Ljubuncic P, Dakwar S, Portnaya I, Cogan U, Azaizeh H, Bomzon A. Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. Evid-Based Compl. Alt. 3: 329-338 (2006)
11. Hasani-Ranjbar S, Nayebi N, Larijani B, Abdollahi M. A systematic review of the efficacy and safety of *Teucrium* species; From anti-oxidant to antidiabetic effects. Int. J. Pharmacogn. 6: 315-325 (2010)
12. Menichini F, Conforti F, Rigano D, Formisano C, Piozzi F, Senatore F. Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. Food Chem. 115: 679-686 (2009)
13. Kamel A, Sandra P. Gas chromatography–mass spectrometry analysis of volatile oils of two *Teucrium polium* varieties. Biochem. Syst. Ecol. 22: 529-532 (1994)
14. Piozzi F, Bruno M, Rosselli S, Maggio A. Advances on the chemistry of furano-diterpenoids from *Teucrium* genus. Heterocycles 65: 1221-1234 (2005)
15. Perez-Alonso MJ, Velasco-Negueruela A, Lopez-Saez JM. The essential oils of two Iberian *Teucrium* species. J. Essent. Oil Res. 5: 397-402 (1993)
16. Glasby JS. Dictionary of Plants Containing Secondary Metabolites. CRC Press New York, NY, USA. pp. 318-326 (1991)
17. Rizk AM, Hammouda FM, Rimpler H, Kamel A. Iridoids and flavonoids of *Teucrium polium* herb. Planta Med. 52: 87-88 (1986)
18. Wassel G, Ahmed SS. Chemical composition of the wild Egyptian plant *Teucrium polium*. Pharmazie 29: 351-352 (1974)
19. Barrachina MD, Bello R, Martinez-Cuesta MA, Esplugues J, Primo-Yufera E. Anti-inflammatory activity and effects on isolated smooth muscle of extracts from different *Teucrium* species. Phytother. Res. 9: 368-371 (1995)
20. Bellomaria B, Arnold N, Valentini G. Essential oil of *Teucrium flavum* subsp. *Hellenicum* from Greece. J. Essent. Oil Res. 10: 131-133 (1998)
21. Panovska TK, Kulevanova S, Gjorgoski I, Bogdanova M, Petrushevska G. Hepatoprotective effect of the ethyl acetate extract of *Teucrium polium L*. against carbontetrachloride-induced hepatic injury in rats. Acta Pharmaceut. 57: 241-248 (2007)
22. Saracogluet I, Calis I, Inoue M, Ogihara Y. Selective cytotoxic and cytostatic activity of some phenylpropanoid glycosides. Fitoterapia 68: 434-438 (1997)
23. Pottier A. Flora of Tunisia Angiosperms Broadleaf apetalous-Dialypetalous. Program Tunisian flora and vegetation. Official Printing Office of the Republic of Tunisia. Tunis, Tunisia. pp. 269-328 (1979)
24. Kovàts E. Characterization of organic compounds by gas chromatography. Part 1. Retention indices of aliphatic halides, alcohols, aldehydes and ketones. Helv. Chim. Acta. 41**:** 1915-1932 (1958)
25. Folin O, Ciocalteu V. On tyrosine and tryptophane determination in proteins. J. Biol. Chem. 73: 627-650 (1927)
26. Li H, Cheng K, Wong C, Fan K, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 102: 771-776 (2007)
27. Bahorun CM, Shahidi F. Antioxidant propreties of commercial soft and hard winterwheats (*Triticumaestivium L*.) and their milling fractions. J. Sci. Food Agr. 86: 477-485 (1996)
28. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agr. 76: 270-276 (1998)
29. Oyaizu M. Studies On products of browning reactions: Antioxidative activities of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307–315 (1986)
30. Koleva LI, Teris AB, Jozef PH, Linssen AG, Lyuba N. Screening of plant extracts for antioxidant activity. A comparative study on three testing methods. Phytochem. Analysis 13: 8-17 (2002)
31. Hsu B, Coupar IM, Ng K. Antioxidant activity of hot water extract from the fruit of the Doum palm, *Hyphaene thebaica*. Food Chem. 98: 317-328 (2006)
32. Majorie MC. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12: 564-582 (1999)
33. Mizuno T. Medicinal properties and clinical effects of culinary-medicinal mushroom *Agaricus blazei* Murrill (Agaricomycetideae). Int. J. Med. Mu. Mushrooms 4: 299-312 (2002)
34. Djouahri A, Saka B, Boudarene L, Benseradj F, Aberrane S, Aitmoussa S, Chelghoum C, Lamari L, Sabaou N, Baaliouamer A. *In vitro* synergistic/ antagonistic antibacterial and anti-inflammatory effect of various extracts/ essential oil from cones of *Tetraclinis articulata* (Vahl) Masters with antibiotic and anti-inflammatory agents. Ind. Crop. Prod. 56: 60-66 (2014)
35. Cozzani S, Muselli A, Desjobert JM, Bernardini AF, Tomi F, Casanova J. Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. Flavour Frag. J. 20: 436-441(2005)
36. Moghtader M. Chemical composition of the essential oil of Teucrium polium L. from Iran. Am. Eurasian J. Sci. Res. 5: 843-846 (2009).
37. Belmekki N, Bendimerad N, Bekhechi C, Fernandez X. Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria. J. Med. Plants Res. 7: 897-902 (2013)
38. Kadri A, Zarai Z, Ben Chobba I, Békir A, Gharsallah N, Damak M, Gdoura R. Chemical constituents and antioxidant properties of *Rosmarinus officinalis* L. essential oil cultivated from the South-Western of Tunisia. J. Med. Plants Res. 5: 6502-6508 (2011)
39. Cuendet M, Hostettmann K, Potterat O. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helv. Chim. Acta 80: 1144-1152 (1997)
40. Birasuren B, Kim NY, Jeon HL, Kim MR. Evaluation of the antioxidant capacity and phenolic content of *Agriophyllum pungens* seed extracts from Mongolia. Prev. Nutr. Food Sci. 18: 188-195 (2013)
41. Trabelsi N, Oueslati S, Ksouri R, Nassra BM, Marchal A, Krisa S, Abdelly C, Merillon JM, Waffo-Teguo F. The antioxidant properties of new dimer and two monomers of phenolics acid amides isolated from *Limoniastrum guyonianum.* Food Chem. 146: 466-471(2014)
42. Ozusaglam MA, Derya Onal Darilmaz DO, Erzengin M, Teksen M, Erkul SK. Antimicrobial and antioxidant activities of two endemic plants from Aksaray in Turkey. Afr. J. Tradit. Complement. Altern. Med. 10: 449-457 (2013)
43. Ulubelen A, Topcu G, Sonmez U. Chemical and biological evaluation of genus *Teucrium*. Stud. Nat. Prod. Chem. 23: 591-648 (2000)