**Aqueous Extracts of *Teucrium polium* Possess Remarkable Antioxidant Activity *In Vitro***

Predrag Ljubuncic, Suha Dakwar, Irina Portnaya, Uri Cogan, Hassan Azaizeh and Arieh Bomzon

Department of Pharmacology, Bruce and Ruth Rappaport Faculty of Medicine, Faculty of Biotechnology and Food Engineering, Technion—Israel Institute of Technology, Haifa, Israel and Research and Development Regional Centre (affiliated with Haifa University, Haifa, Israel), The Galilee Society, Shefa Amr, Israel

*Teucrium polium* L. (Lamiaceae) (RDC 1117) is a medicinal plant whose species have been used for over 2000 years in traditional medicine due to its diuretic, diaphoretic, tonic, antipyretic, antispasmodic and cholagogic properties. The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties. We previously reported that an aqueous extract of the leaves and stems of this plant could inhibit iron-induced lipid peroxidation in rat liver homogenate at concentrations that were not toxic to cultured hepatic cells. Others have reported that organic extracts of the aerial components of this plant could inhibit oxidative processes. Against this background, we felt further investigation on the antioxidant action of the extract of *T. polium* prepared according to traditional Arab medicine was warranted. Accordingly, we assessed (i) its ability to inhibit (a) oxidation of β-carotene, (b) 2,2'-azobis(2-amidinopropan) dihydrochloride (AAPH)-induced plasma oxidation and (c) iron-induced lipid peroxidation in rat liver homogenates; (ii) to scavenge the superoxide (O$_2^-$) radical and the hydroxyl radical (OH$^-$); (iii) its effects on the enzyme xanthine oxidase activity; (iv) its capacity to bind iron; and (v) its effect on cell glutathione (GSH) homeostasis in cultured Hep G2 cells. We found that the extract (i) inhibited (a) oxidation of β-carotene, (b) AAPH-induced plasma oxidation and (c) Fe$^{2+}$-induced lipid peroxidation in rat liver homogenates (IC$_{50}$ = 7 ± 2 µg ml$^{-1}$); (ii) scavenged O$_2^-$ (IC$_{50}$ = 12 ± 3 µg ml$^{-1}$) and OH$^-$ (IC$_{50}$ = 66 ± 20 µg ml$^{-1}$); (iii) binds iron (IC$_{50}$ = 79 ± 17 µg ml$^{-1}$); and (iv) tended to increase intracellular GSH levels resulting in a decrease in the GSSG/GSH ratio. These results demonstrate that the extract prepared from the *T. polium* possesses antioxidant activity *in vitro*. Further investigations are needed to verify whether this antioxidant effect occurs *in vivo*.

**Keywords:** *Teucrium polium* – antioxidant – superoxide radical – hydroxyl radical – glutathione

---

**Introduction**

The medicinal use of plants dates back to ancient times. *Teucrium polium* L. (Lamiaceae) (RDC 1117) is a plant that has been used for over 2000 years in traditional medicine due to its diuretic, diaphoretic, tonic, antipyretic, antispasmodic and cholagogic properties (1–3). In addition, the plant possesses hypoglycemic, insulinotropic and anti-inflammatory activities (4–6), reduces body weight and lowers high blood pressure (7,8) and has hypolipidemic, antinociceptive and antioxidant properties (9–11).

The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties (12–15). In this regard, a methanolic extract of *T. polium* protected red blood cells (RBCs) against lipid peroxidation induced by 10 mM hydrogen peroxide (16). In another study, Kadiskova-Panovska and co-workers (17) demonstrated that extracts of *T. polium* prepared using different organic solvents (diethyl ether, ethyl acetate and n-butanol) were effective inhibitors of β-carotene oxidation. In a previous study, we showed that the
aqueous extracts prepared from the foliage of T. polium suppressed iron (Fe$^{2+}$)-induced lipid peroxidation in rat liver homogenates to the same extent as Trolox, the water-soluble analog of vitamin E (18). Additionally, we showed that the extract was not cytotoxic because it did not adversely affect cell membrane integrity or suppress mitochondrial respiration of cultured Hep G2 and PC12 cells following 24 h exposure (18). In another study, we reported that adding fertilizer caused a significant concentration-dependent increase in antioxidant activity of the cultivated T. polium compared with the wild-type (19).

Based on these findings, we felt further investigation on the antioxidant action of the aqueous extract of T. polium prepared according to traditional Arab medicine was warranted. In this report, we present our findings on the effects of increasing concentrations up to 1 mg ml$^{-1}$ of aqueous extracts prepared from the leaves and stems of T. polium on (i) inhibition of oxidative processes, namely, inhibition of oxidation of β-carotene and plasma oxidation and Fe$^{2+}$-induced lipid peroxidation; (ii) its ability to scavenge reactive oxygen species (ROS), namely the superoxide anion (O$_2^-$) and the hydroxyl radical (OH$^-$); (iii) the activity of superoxide-generating enzyme xanthine oxidase (XO); (iv) its capacity to bind iron; and (v) cell glutathione (GSH) homeostasis.

**Methods**

**Plant Material and Powder Preparation**

Leaves and stems of T. polium L. (Lamiaceae) (RDC 1117) were collected during the spring (May–June) from the hills of the Galilee region of Israel. After collection, plant parts were dried for 7–10 days in the shade at room temperature. They were then ground and the powder was stored in cloth bags at 4°C until required. The resulting filtrate was freeze-dried and the powder was stored at −18°C in a desiccant until required.

**Chemicals and Reagents**

All the materials for the experiments involving cultured cells were purchased from Biological Industries Ltd, Beit Haemek, Israel. All chemicals and reagents of the highest purity were purchased from the Sigma Chemical Co., St Louis, MO, USA.

**Analytical Procedures and Experimental Protocols**

**Does T. polium Inhibit β-Carotene Oxidation?**

The ability of T. polium extract to suppress β-carotene oxidation was assayed using the coupled oxidation of β-carotene and linoleic acid developed by Marco (20) and subsequently modified by Miller (21) and Pratt and Miller (22). The assay measures the rate of consumptive oxidation of β-carotene at 50°C. Two minor assay modifications were made as follows: the plant powder was dissolved in water instead of alcohol to a final concentration of 100 μg ml$^{-1}$ and spectrophotometric readings were taken at 454 nm. The OD$_{344}$ readings of the rate of β-carotene bleaching were recorded at 20 min intervals for 2 h against water blank. The antioxidant butylated hydroxyanisole (BHA) (100 μg ml$^{-1}$) served as a positive control. The assay was repeated three times.

**Suppression of 2,2′-Azobis(2-aminopropane)dihydrochloride-Induced Plasma Oxidation**

The assay relies on ability of the hydrophilic radical generator 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH) to generate peroxy radicals, thereby oxidizing plasma (23,24) and measuring the extent of plasma oxidation by the thiobarbituric acid reactive substances (TBARS) assay (25). Human plasma from healthy donors, obtained from the Haifa branch of Israel Blood Bank, was diluted (1:4) with phosphate-buffered saline (PBS). Aliquots (2 ml) of diluted plasma were incubated with freshly prepared 50 mM AAPH in the absence (control) and presence of different concentrations of T. polium extract (133, 333 and 667 μg ml$^{-1}$, final concentrations) at 37°C for 2 h. BHA (100 μg ml$^{-1}$) was used as a positive control. At the end of incubation period, the samples were rapidly cooled to stop the plasma oxidation. The generated malondialdehyde (MDA) was measured by the TBARS assay (see later for details). Results were expressed in terms of spectrophotometric readings of the samples at OD$_{532}$ against water blank. The assay was done three times.

**Testing for Iron-Induced Lipid Peroxidation in Liver Homogenates**

The antioxidant activity of the extract was evaluated by quantifying the ability of different concentrations of plant extract to suppress iron (Fe$^{2+}$)-induced lipid peroxidation in rat liver homogenates. Liver homogenates were prepared from male Sprague–Dawley rats weighing between 250 and 300 g scheduled to be killed by decapitation as ‘healthy untreated controls’ in institute-approved animal-based investigations. Briefly, this involved excision of the liver, its washing in ice-cold 0.15 M saline and cutting into small pieces using scissors before homogenization in ice-cold 0.1 M phosphate buffer, pH 7.4, at 4°C with a Potter–Elvehjem glass homogenizer. The resultant homogenate was filtered through four layers of wet gauze and the filtrate collected. The protein concentration of the homogenate was determined by the method described by Lowry and co-workers (26) using bovine serum albumin as a standard. The final protein concentration was adjusted to 10 mg protein per ml.

Aliquots of liver homogenates were incubated with 1 μg ml$^{-1}$ to 1 mg ml$^{-1}$ concentrations of extracts of T. polium in a
The extent of suppression of Fe²⁺ equivalents using 1,1,3,3-tetraethoxypropane as the standard. In the same way to exclude any A₅₃₂ contribution due to the Na₂EDTA, 40 \mu M extracts of X₀ and its rate was continuously monitored spectrophotometrically. The xanthine–xanthine oxidase (X–XO) enzymatic reaction is the hydrophilic analog of vitamin E. The assay was incubated with reducing agent and H₂O₂ in the absence of presence of 5 \mu g to 1 mg T. polium extract. The reaction was commenced by adding 100 \mu M ascorbic acid (AA). The samples were incubated for 60 min at 37°C in shaking water bath followed by the TBARS assay. The yield of inhibition was compared with equivalent data obtained using 1 mM mannitol, the well-known OH• radical scavenger. The assay was repeated eight times. Prior to execution of each assay, the stability of the H₂O₂ solution was checked spectrophotometrically using the equation that 10 mM H₂O₂ solution gives an OD reading of 0.436 absorbance units at 240 nm.

**Superoxide Anion Scavenger Potential**

The xanthine–xanthine oxidase (X–XO) enzymatic reaction is a suitable system to generate superoxide anion (O₂⁻) (27). Using this reaction, we evaluated the ability of 1 \mu M to 1 mg ml⁻¹ extracts of T. polium to scavenge O₂⁻ using the nitroblue tetrazolium (NBT) reduction assay as described by Aruoma et al. (1989). The reaction mixture contained 100 \mu M Na₂EDTA, 40 \mu M X and 40 \mu M NBT in 10 mM phosphate buffer pH 7.4. The reaction was started by adding 10 \mu M ml⁻¹ XO and its rate was continuously monitored spectrophotometrically at OD₅₃₀ nm for 15 min at 25°C in the absence and presence of the different concentrations of T. polium extracts. The specificity of the reaction was confirmed using 300 \mu M ml⁻¹ superoxide dismutase (SOD). The ability of the extracts to scavenge O₂⁻ was expressed as percent inhibition of NBT reduction in Teucrium-present samples compared to NBT reduction in Teucrium-absent samples. Four duplicate measurements were done at each concentration of extract.

**Influence on the Activity of XO**

Compounds that interact with XO can affect the kinetics of reaction of oxidation of xanthine to uric acid (28). Accordingly, we assessed the effect of 1 \mu M ml⁻¹ to 1 mg ml⁻¹ extracts of T. polium on XO activity by spectrophotometrically monitoring the rate of uric acid formation at OD₃₉₅ nm for 3 min at 25°C in the absence and presence of extracts using the method described by Marcocci et al. (29). The rate of uric acid formation was compared in the absence and presence of extracts. The specificity of the reaction was confirmed using 100 \mu g ml⁻¹ allopurinol. In this experiment, duplicate measurements were done five times at each concentration.

**Hydroxyl Radical Scavenger Potential**

The ability of T. polium to scavenge OH• radical was assessed using the classic deoxyribose degradation assay described by Halliwell et al. (30). When EDTA chelated iron-(III) ions are incubated with reducing agent and H₂O₂ in the assay, OH• radicals are generated in free solution that attack the deoxyribose substrate and fragmenting it into TBARS. The generated TBARS reflect the extent of generation of OH•. OH• is generated in a 1 ml reaction mixture containing 0.1 mM phosphate buffer, pH 7.4, 2.8 mM deoxyribose, 20 \mu M FeCl₃, 100 \mu M EDTA (premixed immediately before its addition to the reaction mixture) and 500 \mu M H₂O₂ in the absence or presence of 5 \mu g to 1 mg T. polium extract. The reaction was commenced by adding 100 \mu M ascorbic acid (AA). The samples were incubated for 60 min at 37°C in shaking water bath followed by the TBARS assay. The extent of inhibition was compared with equivalent data obtained using 1 mM mannitol, the well-known OH• radical scavenger. The assay was repeated eight times. Prior to execution of each assay, the stability of the H₂O₂ solution was checked spectrophotometrically using the equation that 10 mM H₂O₂ solution gives an OD reading of 0.436 absorbance units at 240 nm.

**Can T. polium Bind with Iron?**

The deoxyribose degradation assay can also be used to assess the ability of a compound to bind iron when the assay is performed in the absence of EDTA (31,32). When FeCl₃ is added to the deoxyribose assay in the absence of EDTA, Fe³⁺ reacts with deoxyribose generating OH• radicals that immediately attack the deoxyribose. Any molecule that can prevent the process of deoxyribose degradation under such conditions are those that bind iron in weakly reactive forms to generate OH• radicals. Accordingly, incubations were done in the absence and presence of 5 \mu g to 1 mg T. polium in the deoxyribose assay system with the omission of EDTA followed by the TBARS assay. The extent of inhibition was compared with equivalent data obtained using 1 mM mannitol. The experiment was repeated seven times.

**Changes in Cellular GSH Content and its Redox State**

The effects of T. polium extracts on GSH content and redox state were assessed in cultured Hep G2 cells. Hep G2 cells are derived from a human hepatoblastoma and retain many of the differentiated features of mature hepatocytes (33). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) with a high glucose content (4.5 g ¹⁻¹) supplemented with 10% vol/vol inactivated fetal calf serum, 1% non-essential amino acids, 1% glutamine, 100 U ml⁻¹ penicillin and 10 \mu g ml⁻¹ streptomycin, pH 7.4, and maintained in a humidified atmosphere of 95% O₂:5% CO₂ at 37°C. In order to assess
the effects of the extract on cellular GSH content and redox state, the cells were seeded and grown in cell flasks under the already described conditions. When 70–80% confluence was reached, the cells were trypsinized, centrifuged (250 g for 5 min at 40°C), resuspended in fresh medium and plated in 6-well dishes (106 cells ml⁻¹). After attachment, the medium was replaced and cells were incubated in fresh serum-free medium containing 10 μg ml⁻¹ to 1 mg ml⁻¹ extract for 24 h at 37°C. At the end of incubation period, the cells were washed three times with ice-cold physiological buffered saline. After washing, cells were scraped into 1 ml of ice-cold PBS. To extract cellular GSH, the cells were sonicated at medium speed with three 20 s bursts and a small part of was taken for protein determination (26). The rest of sonicate was immediately acidified with 5% sulfosalicylic acid (2:1 v/v) to prevent spontaneous oxidation of GSH. After standing for 10 min on ice, the sonicate was centrifuged in a refrigerated tabletop centrifuge at 10 000 g for 10 min to remove denaturated proteins. The resultant protein-free supernatants were transferred into 1.7 ml plastic tubes and the acidified samples were frozen at −70°C for a maximum of 1 week. The non-treated cells served as control.

Determination of GSH levels was performed using the DTNB–GSSG reductase recycling assay described by Tietze (34) with minor modifications. Before measurement of GSH, the sample was thawed and back titrated to pH 7 with 0.2 N NaOH. The GSH content (the sum of the reduced and oxidized forms of GSH) and the oxidized form (GSSG) were measured separately. The GSH assay was performed in a cuvette containing 0.01 M sodium phosphate buffer (pH 7.5), 5 mM EDTA, 10 mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), 2 mM NADPH, 10 U ml⁻¹ GSSG reductase and sample corresponding to ~100 μg of cell protein in a final volume of 1.0 ml. The reaction kinetics was followed spectrophotometrically at OD₄₁₂ nm for 5 min by monitoring increase in absorbance. For determination of GSSG content, the same DTNB–GSSG reductase recycling assay was performed after the alkylation of the SH groups of reduced GSH by 10 mM N-ethylmaleimide (NEM) to remove reduced GSH from the reaction. To avoid the adverse effects of excess NEM on the assay used to determine GSSG levels, excess NEM was removed by separation on a Sep-Pak (C18) column (Sigma Chemical Corp., MO, USA). The increments in absorbance at OD₄₁₂ nm were converted to GSH and GSSG concentrations using a standard curve (0–2.5 nmol GSSG). Duplicate GSH and GSSG determinations were repeated three times. The results were expressed in nmol GSH per mg protein.

**Statistical Analysis**

In order to calculate the concentration at which the plant inhibited Fe²⁺-induced lipid peroxidation or scavenge O₂⁻, OH⁻ radicals and bind iron by 50%, data relating the ability of increasing concentrations of the extract in these four assays were analyzed by plotting effect versus concentration using the Figure Perfect statistical package (Fig.P®; Biosoft, Cambridge, UK) using a standard curve-fitting program. The correlation between the model and the data of the individual concentration response curves was always greater than 0.93. All data were analyzed by repeated measures one-way analysis of variance (ANOVA) with Dunnett’s post-test using Instat™ version 3 (GraphPad Software Inc., San Diego, CA, USA). Significance was set at 5%.

**RESULTS**

**Decreased Rate in Oxidation of β-carotene**

Initially, we evaluated the antioxidant potential of the extract by determining the ability of 100 μg ml⁻¹ extract to inhibit β-carotene oxidation. The rate of β-carotene oxidation was reduced by 60.0 ± 2.4% at this concentration, whereas BHA almost completely inhibited the rate of β-carotene oxidation (Fig. 1, upper).

**Inhibition of AAPH-Induced Plasma Oxidation**

Increasing concentrations of the extract cause a significant concentration-dependent inhibition of AAPH-induced plasma oxidation reaching 84% at the highest concentration (Fig. 1, lower).

**Iron-Induced Lipid Peroxidation Prevented in Liver Homogenate**

The addition of 100 (M FeSO₄ to liver homogenates for 1 h caused almost a 30-fold increase (P < 0.001) in the extent of lipid peroxidation compared to the control samples (0.20 ± 0.15 nmol MDA per mg protein versus 5.41 ± 0.87 nmol MDA per mg protein) (Fig. 2). When different concentrations of the extract was added to the Fe²⁺-containing homogenates, we observed a significant concentration-dependent inhibition of the extent of lipid peroxidation. At concentrations of the extract >50 μg ml⁻¹, inhibition of the extent of lipid peroxidation was 90% and equal to the inhibition obtained using 1 mg ml⁻¹ Trolox. The calculated IC₅₀ value of the response curve was 7 ± 2 μg ml⁻¹ (Fig. 2, inset).

**The Excellent O₂⁻ Scavenger of T. polium**

Figure 3 (upper) summarizes the experiments whose aim was to establish whether the extract contained an O₂⁻ scavenger using the NBT reduction assay. It can be seen that the extract inhibited the rate of NBT reduction in a concentration-dependent manner. The calculated IC₅₀ value of the response curve was 12 ± 3 μg ml⁻¹. To eliminate the possibility that the extract suppressed the rate of conversion of X to uric acid to account for this O₂⁻ scavenging ability, we also measured the effects of the identical concentrations of the extract on XO activity (Fig. 3, lower). We observed significant inhibition of activity only at the three highest concentrations (0.25, 0.5 and 1 mg ml⁻¹) with 1 mg ml⁻¹ concentration causing complete inhibition.
D-ribose is Protected Against OH·/C15-induced Degradation

Increasing concentrations of extracts scavenge OH· in a concentration-dependent manner (Fig. 4, upper). The calculated IC₅₀ value of the response curve was 36 ± 20 μg ml⁻¹ (Fig. 4, upper, insert).

Is D-ribose Degradation Prevented by Iron Binding?

When we used the deoxyribose degradation assay in the absence of EDTA to assess the ability of the extracts to bind iron, we detected a concentration-dependent effect (Fig. 4, lower). The calculated IC₅₀ value of the response curve was 79 ± 17 μg ml⁻¹ (Fig. 4, lower, insert).
Reduced GSH Concentrations Increase in Cultured Hepatocytes

Figure 5 (upper) shows the effects of the plant extract on the GSH levels in Hep G2 cells. The extract did not have any significant effect on GSH levels except at its highest concentration (1 mg ml\(^{-1}\)) where GSH content was five times higher than the GSH content in untreated cells. The effect on GSSG content (Fig. 5, middle). When the GSSG/GSH ratio was calculated, we observed a concentration-dependent decrease in this ratio that was statistically non-significant (Fig. 5, lower).

Discussion

In a previous study, we found that an aqueous extract prepared from the aerial components of \textit{T. polium}, according to the time-honored method of preparing a decoction in traditional Arabic medicine, was very effective in suppressing iron-induced lipid peroxidation (18). In the present study, we extended this observation to further investigate its antioxidant properties using a variety of cell and cell-free in vitro assays. In this regard, we found that the extract (i) inhibited (a) oxidation of \(\beta\)-carotene, (b) AAPH-induced plasma oxidation and (c) \(\text{Fe}^{2+}\)-induced lipid peroxidation in rat liver homogenates; (ii) scavenged \(\text{O}_{2}^{-}\) and \(\text{OH}^{\cdot}\); (iii) bound free iron; and (iv) tended to increase intracellular GSH levels resulting in a decrease in the GSSG/GSH ratio.

Our results are unequivocal in establishing that an aqueous extract of \textit{T. polium} can effectively inhibit oxidative processes. This finding was based on a series of experiments involving three different antioxidant assays, namely oxidation of \(\beta\)-carotene, AAPH-induced plasma oxidation and \(\text{Fe}^{2+}\)-induced...
lipid peroxidation in rat liver homogenates in which we were able to progressively refine the relationship between the ability of the extract to inhibit an oxidative process and its concentration. Previously, we reported that the IC50 of the extract to inhibit Fe2⁺-induced lipid peroxidation in rat liver homogenates was 16 ± 2 μg ml⁻¹ (18). In the present experiment using the identical assay, we established that the IC50 of the extract was 7 ± 2 μg ml⁻¹ with maximal inhibition (90%) already evident at the 0.1 mg ml⁻¹ extract concentration. One may ascribe the different IC50 values to the current study using a different batch of collected plant material for the powder preparation, albeit using the same method of preparation.

We also found that the extract is an efficient scavenger of O₂⁻. The extract concentration at which 50% inhibition of NBT reduction occurred was 12 ± 3 μg ml⁻¹. This IC50 value is considerably lower than concentrations at which the extract inhibits the activity of XO and similar to the concentration at which the extract inhibited Fe2⁺-induced lipid peroxidation in rat liver homogenates. In a second series of experiments involving the deoxyribose degradation assay, we found that the extract was capable of scavenging OH⁺ and binding free iron. The IC50 concentrations of the extract calculated when using these two assays were similar [66 ± 20 μg ml⁻¹ (non-site-specific deoxyribose assay) and 79 ± 17 μg ml⁻¹ (site-specific deoxyribose assay)]. From these results, it is evident that scavenging OH⁺ and iron chelation account for the antioxidant mechanism whereby the extract protects against deoxyribose degradation. Our experiments also involved evaluation of the extract on GSH homeostasis in cultured

Figure 4. The ability of increasing concentrations of extracts of T. polium to scavenge OH⁺ radical using the deoxyribose assay (upper) and to bind iron using deoxyribose degradation assay in the absence of EDTA (lower). Data are shown as mean ± standard deviation. ***P < 0.001 and represents the significance of the difference from the control. n (sample size) = 7–8.
hepatocytes. In these experiments, we observed that the extract tended to increase reduced GSH levels without affecting GSSG levels resulting in a decrease in the GSSG/GSH ratio. These results raise the possibility that this plant may increase the intracellular antioxidant levels. Further experiments on cellular GSH homeostasis are needed to verify this suggestion.

Our results are in agreement with those reported by other groups of investigators using a variety of other assays to assess the antioxidant activity of extracts of *T. polium* (11,16,17). In their study, Couladis and her co-workers (11) screened Greek aromatic plants from the Lamiaceae family for the antioxidant activity. Of the 21 plants tested, they found ethanol extracts prepared from *T. polium*, as well as 9 other plants, exhibited the same antioxidant activity as α-tocopherol in their ability to inhibit bleomycin-Fe(II) complex-induced arachidonic acid superoxidation to MDA. Regrettably, these authors did not include any data about the plant extract concentration(s) in their report. In another study, Suboh and co-workers (16) investigated the antioxidant activity effects of various concentrations of methanol extracts of *T. polium* and the six other medicinal plants as high as 1 mg ml⁻¹, the identical concentration to the highest one used in our study. They

---

**Figure 5.** The effect of increasing concentrations of extracts of *T. polium* on reduced GSH (black columns) and GSSG (white columns) (upper) and the GSSG/GSH ratio (lower). Data are shown as mean ± standard deviation. ***P < 0.001 and represent the significance of the difference from the control. n (sample size) = 3.
reported that the extract significantly reduced 10 mM hydrogen peroxide-induced lipid peroxidation in human erythrocytes. In contrast, the extract failed to protect erythrocytes against protein oxidation and loss of cell deformability of the oxidatively stressed erythrocytes. Kadifkova-Panovska and co-workers (17) also investigated the antioxidant activity of extracts of T. chamaedrys, T. montanum and T. polium prepared using different organic solvents, namely diethyl ether, ethyl acetate and n-butanol. Using such extracts, they assessed their antioxidant abilities in a series of assays that included inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH assay), inhibition of hydroxyl radical (D-ribose assay) and inhibition of β-carotene oxidation. They found that a 0.4 mg ml\(^{-1}\) extract of T. polium was very effective in inhibiting β-carotene oxidation. Based on our results and these reports, there can be little doubt that extracts of T. polium, irrespective of the mode of preparation, can inhibit oxidative processes leading us to conclude that the extract has substantial antioxidant activity in vitro. We did not attempt to identify the chemical constituents that could account for the antioxidant action of the extract. However, other investigators have reported that the aerial parts of T. polium can be attributed to presence of these bioactive components.

In our previous study, we reported that an aqueous extract of T. polium was not toxic to cultured hepatic cells since mitochondrial respiration was fully preserved and the cell membrane integrity remained intact when cells were exposed for 24 h to the plant powder up to 1 mg ml\(^{-1}\) concentration (17). Based on the results of this study and our previous report, we conclude that an aqueous extract of T. polium possesses remarkable antioxidant activities at the concentrations that are not toxic to cultured hepatic cells. Accordingly, we propose that the therapeutic benefit of the extract as used in traditional Arab medicine might be due to its antioxidant activity. Further investigations are needed to verify whether this antioxidant effect occurs in vivo.

Acknowledgements

This research was supported by grants from the Ministry of Science and Technology, Jerusalem, Israel, The Center for Absorption in Science of the Ministry of Immigrant Absorption and the Committee for Planning and Budgeting of the Council for Higher Education under the framework of the KAMEA and GILADI Programs and the Technion Vice-President Research Fund for Promotion for Research at the Technion.

References

1. Galai EM, Mondello MR, D’Aquino A, Miceli N, Sanogo R, Tzakou O, et al. Effects of Teucrium divaricatum Heldr. ssp. divaricatum decoction on experimental ulcer in rats. J Ethnopharmacol 2000;72:337–42.
2. Palevič D, Yaniv Z. Medicinal Plants of the Holy Land, Tel Aviv, Israel: Modan Publishing House, 2000.
3. 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 2002;83:251–65.
4. Gharaibaib MN, Elayyan HH, Salhab AS. Hypoglycemic effects of Teucrium polium. J Ethnopharmacol 1988;24:93–9.
5. Tariq M, Ageel AM, al-Yahya MA, al-Said MS. Anti-inflammatory activity of Teucrium polium. Int J Tissue React 1989;11:185–8.
6. Esmaeili MA, Yazdanparast R. Hypoglycaemic effect of Teucrium polium: studies with rat pancreatic islets. J Ethnopharmacol 2004;95:27–30.
7. Gharaibaib MN, Elayyan HH, Salhab AS. Anorexic effect of Teucrium polium in rats. Int J Crude Drug Res 1989;27:201–7.
8. Suleiman MS, Abdul-Ghani AS, Al-Khalil S, Amin R. Effect of Teucrium polium boiled leaf extract on intestinal motility and blood pressure. J Ethnopharmacol 1988;22:111–6.
9. Rasekh HR, Khoshnoosh-Mansourkhani MI, Kamalinejad M. Hypolipidemic effects of Teucrium polium in rats. Fitoterapia 2001;72:937–9.
10. Abdollahi M, Karimpour H, Monsel-Esfehani HR. Antinociceptive effects of Teucrium polium L. total extract and essential oil in mouse writhing test. Pharm Res 2003;48:31–35.
11. Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of some Greek aromatic plants for antioxidant activity. Phytother Res 2003;17:194–5.
12. Hertog MG, Feskens EJ, Hollman PC, Katan MB. Coronhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993;342:1007–11.
13. Zhang Z, Chang Q, Zhu M, Huang Y, Ho WKK, Chen ZY. Characterization of antioxidants present in hawthorn fruits. J Nutr Biochem 2001;12:144–52.
14. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism and bioactivity. Free Radic Biol Med 2004;36:827–8.
15. Dixon RA, Xie DY, Sharma SB. Proanthocyanidins—a final frontier in flavonoid research? New Phytol 2005;165:9–28.
16. Suboh SM, Bilto YY, Aburjai TA. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. Phytother Res 2004;18:280–4.
17. Kadifkova-Panovska T, Kulevanova S, Stefova M. In vitro antioxidant activity of some Teucrium species (Lamiaceae). Acta Pharm 2005;55:207–14.
18. Ljubuncic P, Azaizeh H, Portnaya I, Cogan U, Said O, Saleh KA, et al. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. J Ethnopharmacol 2005;99:43–7.
19. Azaizeh H, Ljubuncic P, Portnaya I, Said O, Cogan U, Bonzon A. Fertilization-induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. Evid Based Complement Alternat Med 2005;2:549–56.
20. Marco GJ. A rapid method for evaluation of antioxidants. J Am Oil Chem Soc 1968;45:594–8.
21. Miller HE. A simplified method for evaluation of antioxidants. J Am Oil Chem Soc 1971;48:91.
22. Pratt DE, Miller EE. A flavonoid antioxidant in Spanish peanuts (Arachita hypogaea). J Am Oil Chem Soc 1984:61:1064–7.
23. Frei B, Gazzano JM. Content of antioxidants, preformed lipid hydroperoxides, and cholesterol as predictors of the susceptibility of human LDL to metal ion-dependent and -independent oxidation. J Lipid Res 1993;34:2135–45.
24. El-Saadani M, Esterbauer M, El-Sayed M, Nasser AY, Jurgens JA. A spectrophotometric assay for lipid peroxides in serum lipoproteins using a commercially available reagent. J Lipid Res 1989;30:657–30.
25. Draper HH, Hadley M. Malondialdehyde as a measurement with the Folin phenol reagent. J Biol Chem 1970;245:365–57.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
27. McCord JM, Fridovich I. The reduction of cytochrome c by milk xanthine oxidase. J Biol Chem 1968;243:5753–60.
28. Fridovich I. Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. J Biol Chem 1970;245:4053–7.
29. Marcocci L, Suzuki YJ, Tsuchiya M, Packer L. Antioxidant activity of nitrocatechol and its analog OR-1246: effect of structural modification on antioxidant action. *Methods Enzymol* 1994;234:525–41.

30. Halliwell B, Gutteridge JMC, Auroma OI. The deoxyribose method: a simple ‘test-tube’ assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem* 1987;165:215–9.

31. Evans PJ, Whiteman M, Tredger JM, Halliwell B. Antioxidant properties of S-adenosyl-L-methionine: a proposed addition to organ storage fluids. *Free Radic Biol Med* 1997;23:1002–8.

32. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat Res* 2003;523–524:9–20.

33. Knowles BB, Howe CC, Aden DP. Human hepatocellular carcinoma cell lines secrete the major plasma proteins and hepatitis B surface antigen. *Science* 1980;209:497–9.

34. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 1969;27:502–22.

35. Harborne JB, Tomas B, Williams CA, Gil MI. A chemotaxonomic study of flavonoids from European *Teucrium* species. *Phytochemistry* 1986;25:2811–6.

36. Rizk AM, Hammouda FM, Rimpler H, Kamel A. Iridoids and flavonoids of *Teucrium polium* herb. *Planta Med* 1986;2:87–8.

Received September 12, 2005; accepted May 25, 2006