Antioxidant and Antiacetylcholinesterase Activity of *Teucrium hyrcanicum*

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ABSTRACT

Background: *Teucrium hyrcanicum* belonging to the Lamiaceae family is a native plant in Iran; it is called Maryam nokhodi-e-jangali in Farsi. **Objective:** The aim of this study is to evaluate acetylcholinesterase inhibition (AChEI), antioxidant activity and flavonoids content of *T. hyrcanicum* methanol extract. **Materials and Methods:** The air-dried and the ground aerial parts of *T. hyrcanicum* were extracted by percolation method with methanol. Antioxidant activity of the extract was investigated by using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assay (FRAP) methods. In addition, AChEI and flavonoid content of *T. hyrcanicum* methanol extract were measured. **Results:** The results showed that total flavonoid content of *T. hyrcanicum* in reference to the standard curve for quercetin was 20.70 ± 0.05 mg quercetin equivalents/g of extract. In the FRAP method, the antioxidant activity of *T. hyrcanicum* extract and butyl hydroxyanisole (BHA) (as a positive control) were 657.5 ± 0.04 and 880 ± 0.06 mmol Fe II/1 g dried extract. According to results of DPPH assay, half maximal inhibitory concentration (IC_{50}) value for DPPH radical-scavenging activities of *T. hyrcanicum* methanol extract, vitamin E and BHA were 74.6, 14.12 and 7.8 µg/mL, respectively. IC_{50} value for AChEI of *T. hyrcanicum* and donepezil as a positive control were 2.12 mg/mL and 0.013 mg/mL. **Conclusion:** The results of the present study showed *T. hyrcanicum* is a natural antioxidant that the flavonoid content can be responsible for extract effects.

Key words: Acetylcholinesterase inhibitory, antioxidant activity, flavonoid contents, *Teucrium hyrcanicum*

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INTRODUCTION

Alzheimer's disease (AD) is the important degenerative disease of the brain among the elderly.[1,2] Studies suggest that AD is caused by reduced levels of acetylcholine.[3] Thus, the use of acetylcholinesterase enzyme inhibitors (AChEIs) is a major treatment option for AD.[4] Some synthetic AD drugs exhibit several side-effects, and many efforts have been made to find natural AChEIs from plants with less adverse effects.[5] Free radicals such as the superoxide anion and hydroxyl and peroxyl radicals, generated from activated neutrophils and macrophages may cause serious diseases including neurodegenerative disorders, cancer and atherosclerosis.[6] Oxidative stress is responsible for cellular damage especially in organs such as the brain.[7,8] Some studies suggest that the brain of an Alzheimer's patient is under oxidative stress resulting from an imbalance of calcium ions within their neurons and mitochondria.[9,10] Siddhuraju (2007) showed herbal products are the natural antioxidants that able to reduce oxidative damage.[11] Some previous studies on the antioxidant activity in plants have focused on phenolic compounds.[12]

Teucrium is a genus belonging to the Lamiaceae family and consists 340 species in the world Among them 12 species exist in Iran and three species are endemic.[13] These species have been used in traditional medicine for their diuretic, diaphoretic, tonic and anti-spasmodic effects.[14] In Iranian and Arab traditional medicine, *Teucrium polium* and *Teucrium persicum* have been used for the treatment of diabetes, gastric

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inflammation and convulsion.[14] In the genus *Teucrium,* flavonoids are important active compounds that attribute to many of the biological effects observed in these species. The quantity of flavonoids can be influenced by various factors. For example, different seasons as well as the locations of plant growth are important to consider in the evaluation of flavonoid content.[15] In one study, *T. polium* subsp. *polium* methanol extract showed antioxidant activity.[16] In another study, flavonoids content of *T. polium* has been reported as 47.80 ± 0.44 mg of rutin equivalent/g of extract.[17]

Teucrium hyrcanicum L. (called Maryam nokhodi-e-jangali in Farsi) is a native plant in Iran which wildly grows in north and northwest of the country.[18] In previous studies, anti-nociceptive and anti-inflammatory activities of *T. hyrcanicum* were investigated.[19] The aim of this study was to evaluate AChEI, antioxidant activity and flavonoids content of *T. hyrcanicum* methanol extract.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. hyrcanicum* were collected from Ardebil province in North-West of Iran, in June 2012. The plant was identified by Dr. Yousef Ajani and given herbarium specimen number (M. Khanavi 1446). The voucher specimen was deposited in Herbarium of Institute of Medicinal Plant (ACECR), Tehran, Iran.

Extraction

The aerial parts of *T. hyrcanicum* (500 g) were dried in the shade and powdered. The air-dried and the ground aerial parts of *T. hyrcanicum* were extracted by percolation method with methanol. The extract was dried using a rotary evaporator to give 52.5 g solid residues. The extract was stored in a refrigerator until required.

Total flavonoid content

Total flavonoid content was determined as described by Saeidnia and Gohari.[20] Five microliter of aluminum trichloride ($AlCl_3$) (2% in methanol) was added to 5 mL of extract (0.4 mg/mL). After 10 min, the absorbance of the mixture was measured at 415 nm. Blank sample consists of 5 mL extract and 5 mL methanol without $AlCl_3$. Total flavonoid content was measured by using a standard curve with quercetin (0–100 mg/L). Total flavonoid content was expressed as mg of quercetin as equivalents (QE)/g of extract. Because quercetin has reported from *Teucrium* species, previously such as *Teucrium arduini* L.[21]

Evaluation of antioxidant activity using ferric reducing antioxidant power assay method

The antioxidant activity of extracts were measured by the ferric reducing antioxidant power assay (FRAP) method based on an established protocol.[22] The principle of this method is the reduction of ferric tripyridyl triazine (Fe [III]-TPTZ) complex to the ferrous tripyridyl triazine (Fe [II]-TPTZ), to its colored ferrous form in the presence of antioxidants. The FRAP reagent contained 5 mL of TPTZ (2,4,6-tripyridyl-s-triazine) solution (10 mmol/L) in HCl (40 mmol/L) plus 5 mL of $FeCl_3$ solution (20 mmol/L) and 50 mL of a 0.3 mol/L acetate buffer solution (pH 3.6) that was prepared freshly and warmed at 37°C. Aliquots of 50 µL sample were mixed with 1.5 mL FRAP reagent then were incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For the construction of the calibration curve, five concentrations of $FeSO_4.7H_2O$ (1000, 750, 500, 250, 125 µmol/L) were used, and the absorbance values were measured as sample solutions. Butyl hydroxyanisole (BHA) was used as a positive control. The antioxidant activity of *T. hyrcanicum* methanol extract was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L $FeSO_4$.[23]

2,2′-diphenyl-1-picrylhydrazyl radical-scavenging activity assay

The antioxidant activities of samples were determined by the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging.[24] Different concentrations of sample solutions (1 mL) of methanol were added to DPPH methanol solution (2 ml, 40 µg/mL). After 30 min, the absorbance was measured at 517 nm. Vitamin E and BHA were used as positive controls. Percentage of radical scavenging activity of samples was calculated by using the equation: Inhibition% = $[(A0–As)/A0] \times 100$ that A0 is the absorbance of the control and As is the absorbance of the sample. Half maximal inhibitory concentration (IC_{50}) values (indicate the concentration of the sample (mg/mL), required to scavenge 50% of DPPH) were calculated from graph-plotted against scavenging percentage and extract concentration.

Acetylcholinesterase inhibition

The enzymatic activity was measured as described by Aazza *et al*.[25] with minor modifications. Fifty microliter of buffer 0.1 M (pH = 8) and 25 µL of test compound (or Donepezil as a positive control) were dissolved in methanol at different concentrations and 25 µL of AChE enzyme (0.22 U/ml) (Sigma-Aldrich Chemie, Germany) was added and mixed. After incubation at 37°C for 15 min, 25 µL of 15 mM acetylthiocholine iodide (Sigma-Aldrich Chemie, Germany) and 125 µL of 3 mM 5,5'-dithiobis (2-nitrobenzoic acid) (Sigma-Aldrich Chemie, Germany) were added and the resulting mixture incubated at room temperature for 30 min. Absorbance of the mixture was measured at 405 nm using a microplate reader (ELX808, BioTek, USA). The inhibitory effect of test compound was calculated by comparison to

the negative control: % = [(A0–A1)/A0] × 100 where A0 was the absorbance of the blank sample and A1 was the absorbance of the test sample. Each test was replicated three times. The inhibition of enzyme activity was expressed as IC_{50} (the concentration of the sample, required to inhibit 50% of the enzyme).[26]

Statistical analyses

All data was expressed as mean ± standard deviation. Statistical analysis was performed with One-way analysis of variance, followed by Tukey *post-hoc* test for multiple comparisons. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

In this study, total flavonoid contents, antioxidant activity and AChEI of methanol extract of *T. hyrcanicum* were determined. The extract yield of the plant was determined as 10.5%. The results of antioxidant activity and flavonoid contents of *T. hyrcanicum* are shown in Table 1. The total flavonoid content of *T. hyrcanicum* in reference to the standard curve for quercetin ($y = 0.0231x - 0.1425$, $R^2 = 0.9805$) was 20.70 ± 0.05 mg QE/1 g of extract. The standard curve for quercetin is shown in Figure 1. The obtained results from antioxidant activity using FRAP method expressed as FRA*P* value [Figure 2]. These values represented mmol. Fe II/1 g dried extract. The antioxidant activity of the *T. hyrcanicum* extract and BHA (as a positive control) were 657.5 ± 0.04 and 880 ± 0.06 mmol Fe II/1 g dried extract, respectively. As this result, in FRAP method, *T. hyrcanicum* extract showed better antioxidant activity than BHA. According to results of DPPH assay, IC_{50} value for DPPH radical-scavenging activities of *T. hyrcanicum* methanol extract, vitamin E and BHA were 74.6, 14.12 and 7.8 μg/mL, respectively. The radical scavenging activity of *T. hyrcanicum* at 400 μg/mL was comparable with vitamin E (40 μg/mL) and with BHA (100 μg/mL) ($P > 0.05$). AchEI activity of methanol extract of *T. hyrcanicum* was studied for the first time. IC_{50} value was calculated from graph-plotted inhibition percentage against extract concentration [Figure 3]. The results showed that IC_{50} value for AchEI of *T. hyrcanicum* and donepezil as a positive control were 2.12 mg/mL and 0.013 mg/mL, respectively.

There are reports about flavonoid content and antioxidant activity of other *Teucrium* species like *Teucrium stocksianum* and *T. polium*.[14,27] The previous studies indicated that methanol extract of *T. polium* and two isolated flavonoids (rutin and apigenin) had radical scavenging activity with IC_{50} = 20.1 ± 1.7, 23.7 ± 1.9 and 30.3 ± 2.1 μg/ml, respectively.[17] The results of another study showed that total flavonoid content of *T. polium* in reference to the standard curve for rutin was 47.80 ± 0.44 mg of rutin equivalent/g of extract).[17] In this investigation, *T. hyrcanicum* showed the antioxidant effect and total flavonoid content less than *T. polium*. The phenolic content and other compounds can be responsible for the antioxidant activity of *T. hyrcanicum* extract.

In previous study, the ethanolic extract of *T. arduini*, *Teucrium chamaedrys*, *Teucrium montanum* and *T. polium* showed remarkable AChEI activity above 50% inhibition rate at 1 mg/mL.[28] In another study, AChEI activity of methanol extract of *T. polium* was 39.10% with inhibition rate at 1 mg/L. Inhibitory activity of methanol extract of *T. hyrcanicum* was 40.50% with inhibition rate at 1 mg/mL. The results of previous study indicated that Lamiaceae species containing phenolic and terpenic compounds can affect AChE activity and oxidative stress. Lamiaceae species may be effective in the prevention and therapy of AD and other neurodegenerative disorders.[28]

CONCLUSION

The results of the present study indicated that *T. hyrcanicum* collected from North-West of Iran is a natural antioxidant that able to reduce oxidative damage. The flavonoid

Table 1: Antioxidant activity and flavonoid contents of *Teucrium hyrcanicum* methanol extract

Sample	FRAP assay[a]	DPPH assay (IC_{50})[b]	Total flavonoids content[c]
Methanol extract of *Teucrium hyrcanicum*	657.5±0.04	74.6	20.70±0.05
BHA (positive control)	880±0.06	7.8	-
Vitamin E (positive control)	-	14.12	-

[a]mmol Fe II/1 g dried extract, [b]μg/mL, [c]as mg QE/1 g of extract. FRAP=Ferric reducing antioxidant power assay; QE=Quercetin equivalents; DPPH=2,2′-diphenyl-1-picrylhydrazyl; BHA=Butyl hydroxyanisole; IC_{50}=Inhibitory concentration

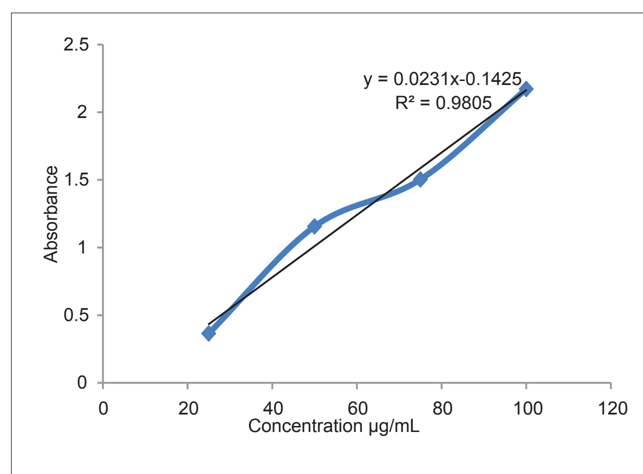

Figure 1: The standard curve of quercetin for measuring total flavonoid content

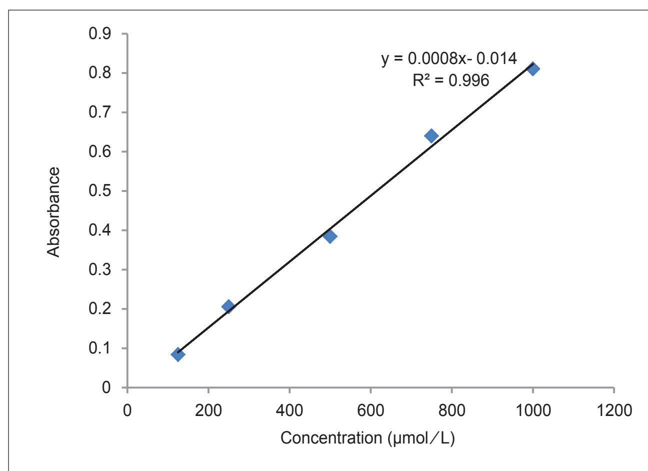

Figure 2: Calibration curve in ferric reducing anti-oxidant power assay method. Five concentrations of FeSO4.7H2O [1000, 750, 500, 250, 125 μmol/L] were used

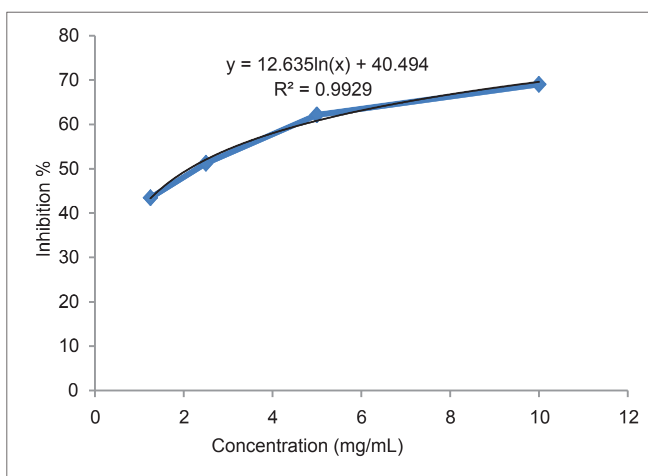

Figure 3: Acetylcholinesterase inhibition activity of methanol extract of *Teucrium hyrcanicum*

content can be responsible for the antioxidant activity of *T. hyrcanicum* extract.

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