

Antioxidant and Antiacetylcholinesterase Activity of *Teucrium hyrcanicum*

Fereshteh Golfakhrabadi¹, Fatemeh Yousefbeyk^{1,2}, Tahmineh Mirnezami¹, Pedram Laghaei³, Mannan Hajimahmoodi^{4,5}, Mahnaz Khanavi^{1,3}

¹Department of Pharmacognosy, Faculty of Pharmacy and Medicinal Plants Research Center, ⁴Department of Drug and Food Control, Faculty of Pharmacy, ⁵Department of Traditional Pharmacy, Faculty of Traditional Medicine and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran 14155 - 6451, ²Department of Pharmacognosy, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran, ³Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada

Submitted: 05-10-2014

Revised: 13-11-2014

Published: 02-06-2015

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 μ g/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*

Access this article online

Website:

www.phcogres.com

DOI: 10.4103/0974-8490.157993

Quick Response Code:



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 peroxy 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

Address for correspondence:

Dr. Mahnaz Khanavi, Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14155 - 6451, Iran.
E-mail: khanavim@tums.ac.ir

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 wildy 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 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1000, 750, 500, 250, 125 $\mu\text{mol/L}$) 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 $\mu\text{g/mL}$). 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: $\text{Inhibition\%} = [(A_0 - A_s) / A_0] \times 100$ that A_0 is the absorbance of the control and A_s 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: % = $[(A_0 - A_1)/A_0] \times 100$ where A_0 was the absorbance of the blank sample and A_1 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 \pm 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 FRAP 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 μ g/mL, respectively. The radical scavenging activity of *T. hyrcanicum* at 400 μ g/mL was comparable with vitamin E (40 μ g/mL) and with BHA (100 μ g/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 $IC_{50} = 20.1 \pm 1.7$, 23.7 ± 1.9 and 30.3 ± 2.1 μ g/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 <i>Teucrium hyrcanicum</i> | 657.5 \pm 0.04 | 74.6 | 20.70 \pm 0.05 |
| BHA (positive control) | 880 \pm 0.06 | 7.8 | - |
| Vitamin E (positive control) | - | 14.12 | - |

^ammol Fe II/1 g dried extract, ^b μ g/mL, ^cas 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

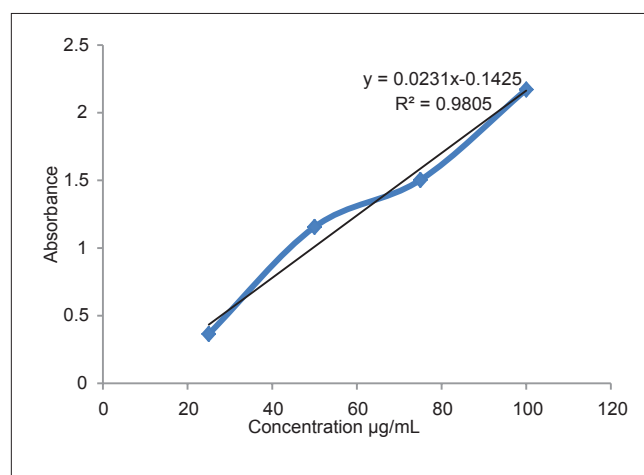


Figure 1: The standard curve of quercetin for measuring total flavonoid content

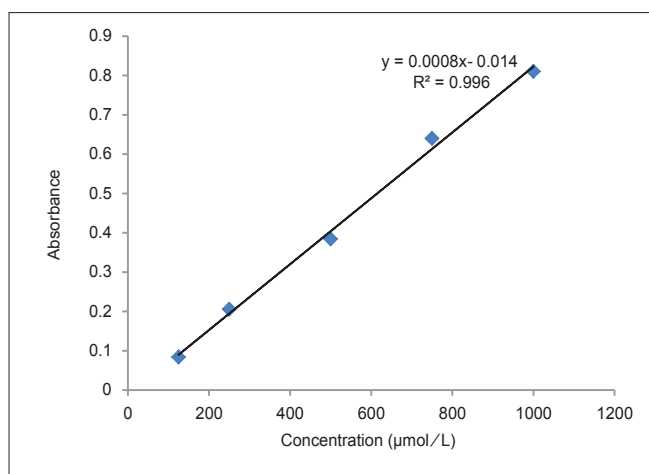


Figure 2: Calibration curve in ferric reducing anti-oxidant power assay method. Five concentrations of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ [1000, 750, 500, 250, 125 µmol/L] were used

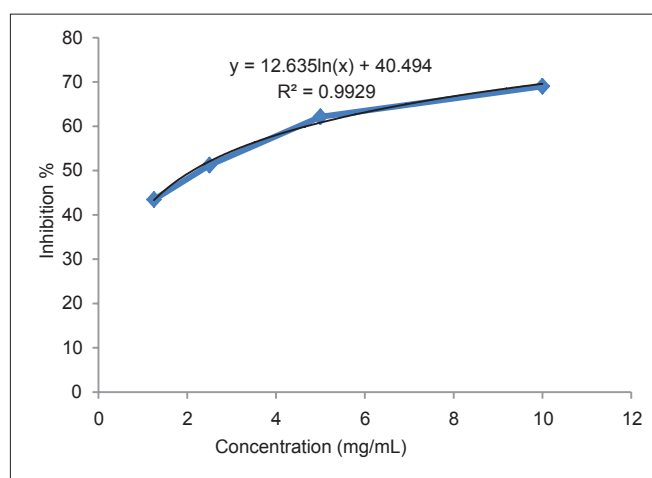


Figure 3: Acetylcholinesterase inhibition activity of methanol extract of *Teucrium hyrcanicum*

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

REFERENCES

- Shen Q, Peng Q, Shao J, Liu X, Huang Z, Pu X, et al. Synthesis and biological evaluation of functionalized coumarins as acetylcholinesterase inhibitors. *Eur J Med Chem* 2005;40:1307-15.
- Zhou X, Wang XB, Wang T, Kong LY. Design, synthesis, and acetylcholinesterase inhibitory activity of novel coumarin analogues. *Bioorg Med Chem* 2008;16:8011-21.
- Weinstock M. Possible role of the cholinergic system and disease models. *J Neural Transm Suppl* 1997;49:93-102.
- Grutzendler J, Morris JC. Cholinesterase inhibitors for Alzheimer's disease. *Drugs* 2001;61:41-52.
- Karimi GH, Iranshahi M, Hosseinalizadeh F, Riahi B, Sahebkar A. Screening of acetylcholinesterase inhibitory activity of terpenoid and coumarin derivatives from the genus *Ferula*. *Pharmacologyonline* 2010;1:566-74.
- Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, et al. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. *J Ethnopharmacol* 2008;116:144-51.
- Halliwell B, Gutteridge JM. Oxygen radicals in the nervous system. *Trends Neurosci* 1985;8:22-6.
- Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol Aging* 2002;23:795-807.
- Emilien G, Beyreuther K, Master CL, Maloteaux JM. Prospects for pharmacological intervention in Alzheimer's disease. *Arch Neurol* 2000;57:454-9.
- Tabet N. Acetylcholinesterase inhibitors for Alzheimer's disease: Anti-inflammatories in acetylcholine clothing! *Age Ageing* 2006;35:336-8.
- Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem* 2007;101:10-9.
- Jafari S, Moradi A, Salaritabar A, Hajiakhoondi A, Khanavi M. Determination of total phenolic and flavonoid contents of *Leonurus cardiac* L. in compare with antioxidant activity. *Res J Biol Sci* 2010;5:484-7.
- Kazemizadeh Z, Habibi B, Moradi A. Chemical composition of the essential oils of two populations *Teucrium hyrcanicum* L. in two different localities. *J Med Plant* 2008;7:87-93.
- Hasani-Ranjbar SH, Nayeibi N, Larijani B, Abdollahi M. A systematic review of efficacy and safety of *Teucrium* species; from anti-oxidant to anti diabetic effects. *Int J Pharm* 2010;6:315-25.
- Steffkov G, Karapandzova M, Stefova M, Kulevanova S. Seasonal variation of flavonoids in *Teucrium polium* L. (*Lamiaceae*). *Macedon Pharm Bull* 2009;55:33-40.
- Stankovic MS, Niciforovic N, Mihailovic V, Topuzovic M, Solujic S. Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*. *Acta Soc Bot Pol* 2012;81:117-22.
- Shariffar F, Dehghn-Nudeh GH, Mitrajaldini M. Major flavonoids with antioxidant activity from *Teucrium polium* L. *Food chem* 2009;112:885-8.
- Rechinger KH. *Flora Iranica*. Vol. 150. Graz: Akademische Druck-u. Verlagsanstalt; 1982.
- Farshchi A, Ghiasi G, Abdollahi Asl A. Antinociceptive and anti-inflammatory effects of *Teucrium hyrcanicum* aqueous extract in male mice and rats. *Physiol Pharmacol* 2010;14:78-84.
- Saeidnia S, Gohari AR. *Pharmacognosy and Molecular Pharmacognosy in Practice: A Laboratory Desk Reference of Pharmacognosy for Researchers and Students*. Saarbrücken, Germany: Lambert Academic Publishing; 2012.
- Kremer D, Kosir IJ, Kosalec I, Koncic MZ, Potocnik T, Cerenak A, et al. Investigation of chemical compounds, antioxidant and antimicrobial properties of *Teucrium arduini* L. (*Lamiaceae*). *Curr Drug Targets* 2013;14:1006-14.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 1996;239:70-6.
- Yousefbeyk F, Gohari AR, Hashemighaderjani Z, Ostad SN, Salehi Sourmaghi MH, Amini M, et al. Bioactive terpenoids and flavonoids from *daucus littoralis* smith subsp. *hyrcanicus* Rech.f, an endemic species of Iran. *Daru* 2014;22:12.
- Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2-picrylhydrazyl radical. *Biochem Pharmacol* 1998;56:213-22.
- Aazza S, Lyoussi B, Miguel MG. Antioxidant and antiacetylcholinesterase activities of some commercial essential

- oils and their major compounds. *Molecules* 2011;16:7672-90.
26. Golfakhrabadi F, Khanavi M, Ostad SN, Saeidnia S, Vatandoost H, Abai MR, *et al.* Biological activities and composition of *Ferulago carduchorum* essential Oil. *J Arthropod Borne Dis* 2015;9:104-15.
 27. Iriadam M, Musa D, Gumushan H, Baba F. Effects of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. *J Cell Mol Biol* 2006;5:19-24.
 28. Vladimir-Knežević S, Blažeković B, Kindl M, Vladić J, Lower-Nedza AD, Brantner AH. Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the *Lamiaceae* Family. *Molecules* 2014;19:767-82.

Cite this article as: Golfakhrabadi F, Yousefbeyk F, Mirnezami T, Laghaei P, Hajimahmoodi M, Khanavi M. Antioxidant and Antiacetylcholinesterase Activity of *Teucrium hyrcanicum*. *Phcog Res* 2015;7:S15-9.

Source of Support: Nil. **Conflict of Interest:** None declared.