Food Chemistry 112 (2009) 885–888

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Major flavonoids with antioxidant activity from *Teucrium polium* L.

Fariba Sharififar [a,*], Gholamreza Dehghn-Nudeh [b], Mansour Mirtajaldini [c]

[a] Kerman University of Medical Science, Department of Pharmacognosy, School of Pharmacy, Haft Bagh Road, Kerman 76195-493, Iran
[b] Kerman University of Medical Science , Pharmaceutics Research Center, Department of Pharmaceutics, School of Pharmacy, Kerman, Iran
[c] International Center for Science and Technology and Environmental Sciences (HITEC), Kerman, Iran

ARTICLE INFO

Article history:
Received 19 April 2008
Received in revised form 17 June 2008
Accepted 26 June 2008

Keywords:
Teucrium polium
Lamiaceae
Flavonoids
Antioxidant
DPPH, beta carotene/linoleic acid
Ammonium thiocyanate

ABSTRACT

Teucrium polium L. (Lamiaceae) aerial parts are used widely in the daily diet and for medicinal purposes. This plant is used also as a spice and refreshing beverage. Phytochemical and bioactivity studies of this plant have been carried out. Aerial parts of the plant were extracted with petroleum ether, chloroform, methanol and water successively. Fractionation of the methanol extract yielded four major flavonoids. The crude extracts and isolated compounds were screened for their antioxidant and free radical scavenging activities using DPPH radical-scavenging, beta-carotene/linoleic acid and ammonium thiocyanate methods. Methanol extract, rutin and apigenin were found to be the most active fractions as radical-scavengers with IC_{50} values of 20.1 ± 1.7, 23.7 ± 1.9 and 30.3 ± 2.1 μg/ml, respectively. The samples with the highest inhibition of oxidation of beta-carotene and lipid peroxidation in ammonium thiocyanate methods were also found to be methanol extract, rutin and apigenin. Methoxylated flavonoids exhibited a lesser antioxidant activity.

1. Introduction

Reactive free radicals, such as superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (·OH), and peroxyl radical (ROO·), are particularly reactive and are known to be a biological product in reducing molecular oxygen (Williams & Jeffrey, 2000). Damages mediated by free radicals result in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions (Kinsella, Frankel, German, & Kanner, 1993), which have generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis. An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases (Shahidi, 1997). The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases (Silva, Souza, Rogez, Rees, & Larondelle, 2007). There is a growing interest in natural antioxidants, present in medicinal and dietary plants that might help attenuate oxidative damage (Silva, Ferreres, Malva, & Dias, 2005). These natural antioxidants not only protect food lipids from oxidation, but may also provide health benefits associated with preventing damages due to biological degeneration (Hu & Kitts, 2005; Shahidi & Wanasundara, 1992). As part of our efforts to find antioxidants from edible herbs, we have investigated the antioxidant potential of *Teucrium polium* L., a plant which belongs to the Lamiaceae. This family is composed of species with exploitable antioxidant activity (del Baño et al., 2003). The genus of *Teucrium* (Lamiaceae) is represented by 12 species in the flora of Iran (Mozaffarian, 1997). *Teucrium polium* L. is one of the wild-growing flowering species from this genus and is found abundantly in Iran. This plant is used to prepare herbal tea and as traditional medicine. The tea of *T. polium* is used as an appetizer especially in children and also as a spice. An infusion of the leaves and flowers of the plant is consumed as a refreshing beverage (Facciola, 1990). The biological activities of *T. polium* is widely reported and it has been shown to possess anti-inflammatory, anti-nociceptive, anti-bacterial, anti-hypertensive, hypolipidemic, anti-rheumatoid, and hypoglycemic effects (Abdollahi, Karimpour, & Monsef-Esfehani, 2003; Rasekh, Khoshnood-Mansourkhani, & Kamalinejad, 2001; Tariq, Ageel, Al-Yahya, Mossa, & Al-Said, 1989). Recently, the high insulinotropic and anti-hyperglycemic activity of its crude extract using both animal and/or isolated rat pancreatic islets has been evaluated (Esmaeili & Yazdanparast, 2004). There are also some reports in the literature for antioxidant effects of crude extract of *T. polium* (Ljubuncic et al., 2006). Two known phenylethanoid glycosides, verbascoside and poliumoside have been reported from the aerial parts of this plant (Bedir, Tasdemi, Çalis, Zerbe, & Sticher, 1999). As far as we know, this is the first report concerning the antioxidant flavonoids of this plant from Iran. In the present work, we wish to report the isolation and structural elucidation of four flavonoids from the aerial parts of *T. polium*, together with their antioxidant and free radical scavenging activity.

* Corresponding author. Tel.: +98 3413220001; fax: +98 3413205201.
E-mail address: fsharififar@Kmu.ac.ir (F. Sharififar).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.064

2. Materials and methods

2.1. Plant materials

Teucrium polium L. was collected from Kerman, Iran in June 2005 and identified by Dr. Mirtajadini, Department of Botany, Bahonar University, Kerman, Iran. A voucher specimen (KF1249) was deposited at the Herbarium of faculty of pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

2.2. Extraction and isolation

The aerial parts of *T. polium* (200 g) were dried and powdered, The powder was passed through a sieve in order to maintain particle size unity (300 μm) and were extracted by percolation method with petroleum ether, chloroform, methanol and water, successively. Solvents were removed by drying at 35 °C in a rotary evaporator prior to storage at 2 °C.

2.3. Antioxidant activity

2.3.1. DPPH assay

Hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-coloured methanol solution of 2,2′-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses stable DPPH radical as a reagent (Burits & Bucar, 2000). Fifty microliters of various concentrations of the samples in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (*I*%) was calculated in the following way:

$$I\% = (A_{<\text{ce:inf}>\text{blank}</\textit{ce:inf}>} - A_{\text{sample}}/A_{\text{blank}} \times 100)$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate.

2.3.2. Beta-carotene bleaching test

Linoleic acid dissolved in ethanol to produce a linoleic acid solution at 2 mg/ml. Linoleic acid solution (10 ml of 2 mg/ml solution in ethanol) and β-carotene solution (10 ml, 2 mg/ml solution im acetone) were added to the molten agar (10 ml, 1.2% solution in boiling water). The mixture was then shaken to give an orange colour. The agar was then poured into Petri dishes (25 ml per dish) and were excluded light and left standing to allow the agar to set. Holes (4 mm diameter) were then punched into the agar and each extract (1 mg) in alcohol were transferred into the holes and the petri dishes were then incubated at 45 °C for 4 h. A zone of colour retention around the hole after incubation indicated sample with antioxidant activities. The zone diameter was measured using vernier calipers. (Graven, Deans, Svoboda, Mavi, & Gundidza, 1992).

2.3.3. Ammonium thiocyanate method (ATC)

The antioxidant activity of the various extracts and flavonoids of *T. polium* were determined using the ATC method (Masude, Isibe, Jitoe, & Naramati 1992). Each sample (500 μg) in 0.5 ml of deionised water was mixed with linoleic acid emulsion (2.5 ml, 0.02 M, pH 7.0 in phosphate buffer) and phosphate buffer (2 ml, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C. Aliquots of 0.1 ml were taken at different intervals during incubation. The degree of oxidation was measured by sequentially adding ethanol (4.7 ml, 75%), an ammonium thiocyanate sample solution (0.1 ml, 30%) and ferrous chloride (0.1 ml, 0.02 M in 3.5% HCl). After 3 min, the peroxide values were determined by reading the absorbance at 500 nm using a spectrophotometer. A control was performed with linoleic acid but without the extracts.

2.4. Separation of the antioxidant flavonoids from the methanol extract

The methanol extract which showed the most antioxidant activity (29.8 g) was fractionated by column chromatography on silica gel 60 (70–230 mesh, Merck; Darmstadt, Germany), using petroleum ether and petroleum ether: chloroform (9:1, 8:2, 5:5, v/v), chloroform, and chloroform: EtOAc (9:1, 8:2, 5:5, v/v), EtOAc and EtOAc: MeOH (9:1, 8:2, 5:5, v/v) as eluents to afford nine fractions (TP1–TP9). Fraction TP4 which showed the most antioxidant activity (2.1 g) was purified by TLC on silica gel 230–400 mesh and eluted with EtOAc: water: formic acid: glacial acetic acid (100:26:11:11, v/v/v/v) afforded four fractions (1–3, 4–8, 9–13, and 14–18). The combined fractions of 14–18 and 9–13 (the most antioxidant active fractions) were purified on Sephadex LH-20 with methanol to afford **1** (0.097 g, 0.33%), **2** (0.070 g, 0.24%), **3** (0.042 g, 0.14%) and **4** (0.034 g, 0.12%).

3. Results and discussion

3.1. Identification of the active flavonoids of methanol extract

Evaporation of the respective solvent gave the petroleum ether (11.8 g, 5.9%), chloroform (7.4, 3.7%), methanol (29.8 g, 14.9%) and aqueous extracts (16 g, 8%). By correlating with melting points and spectral data (UV, IR, ^1H, ^{13}C NMR and MS) of literature values, compounds **1–4** were identified as rutin (**1**), apigenin (**2**), 3′, 6 dimethoxy apigenin (**3**) and 4′,7 dimethoxy apigenin (**4**).

3.2. Antioxidant tests

The relatively stable organic radical, DPPH, has been widely used in the determination of antioxidant activity of single compounds, as well as of different plant extracts (Katalinic, Milos, & Jukic, 2006). There have been extensive studies on antioxidant activity of many spices in the Lamiaceae family. The IC_{50} values for DPPH assay of the samples have been given in Table 1. The methanol extract of *T. polium* was able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to the yellow-coloured DPPH with an IC_{50} of 20.1 ± 1.7 μg/ml, almost near to butylated hydroxytoluene (BHT) (IC_{50} of 18.3 ± 1.9 μg/ml). The isolated flavonoids of rutin and apigenin from this extract also have shown to be active antioxidants (IC_{50} of 23.7 ± 1.9 and 30.3 ± 2.1 μg/ml, respectively). Other samples had antioxidant values between 37.4 and 85.4 μg/ml, measured by the DPPH method. The mechanism of the reaction between antioxidant and DPPH depends on the structural conformation of the antioxidant. Some compounds react very quickly with DPPH, reducing a number of DPPH molecules equal to the number of the hydroxyl groups (Bondet, Williams, & Berset, 1997). The free radical-scavenging activity of flavonoids is dependent on the presence of free OH groups, especially 3-OH. Flavonoids with a 3-OH and 3′,4′-cathecol are reported to be 10-fold more potent than the corresponding catechol and 3-OH free flavonoids (Heim, Tagliaferro, & Bobilya, 2002). In a β-carotene/linoleic acid model system, β-carotene undergoes rapid discoloration in the absence of an antioxidant. Methanol extract, rutin, apigenin and 3′,6-dimethoxyapigenin were able to inhibit the discoloration of beta-carotene with 25.8 ± 1.2, 26.7 ± 1.9,

Table 1
Antioxidant activity of the various extracts and flavonoids from *T. polium*

Sample	DPPH assay IC_{50} (μg/ml)	Beta carotene- linoleic acid mean zone of inhibition (mm)
Petroleum ether ex.	73.2 ± 6.3	9.2 ± 0.7
Chloroform ex.	85.4 ± 7.8	5.1 ± 0.6
Methanol ex.	20.1 ± 1.7	25.8 ± 1.2
Water ex.	40.6 ± 4.0	19.2± 0.8
Rutin	23.7 ± 1.9	26.7 ± 1.9
apigenin	30.3 ± 2.1	27.4 ± 2.2
3′,6-Dimethoxyapigenin	31.5 ± 3.4	20.2 ± 2.5
4′,7-Dimethoxyapigenin	37.4 ± 3.4	18.6 ± 1.5
BHT	18.3 ± 1.9	28.1 ± 2.3

Data represent means ± SD of three independent experiments performed in triplicate.

27.4 ± 2.2 and 20.2 ± 2.2 mm mean zone of colour retention respectively (Table 1). The results of this test corresponded to their free radical scavenging activity of tested fractions. The results of the antioxidant effect of various samples of the plant on the autoxidation of linoleic acid are shown in Figs. 1 and 2. As it is shown, all the tested samples showed antioxidant activity in this system. The methanol extract, rutin and apigenin exhibited the most inhibition of lipid peroxidation. Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic acid and arachidonic acid are the target of lipid peroxidation. Free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation and it is generally thought that the inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Radical scavengers may directly react and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products. The differences in solubility of flavonoids, both aglycones, glycosides, and methoxylated derivatives, in a micellar water–lipid system may influence the results obtained from this test, and the partition of the compounds between the two phases can influence the oxidation results (Burda & Oleszek, 2001). Among the tested samples, the methanol extract showed the most antioxidant activity, which may be attributed to the collective antioxidant effects of other flavonoids present or the other compounds such as phenolics or iridoids which may potentiate the antioxidant activities of flavonoids. These secondary metabolites have been reported in this plant (Proestos, Sereli, & Komaitis, 2006). The presence of rutin and apigenin can explain the high antioxidant effect of methanol extract of the plant. Antioxidant activity of the these two flavonoids have previously been reported (Babenko & Shakhova, 2006; Yang, Guo, & Yuan, 2008). The relationship between the chemical structure of flavonoids and their radical-scavenging activities was analysed by Ye-ilada, Tsuchiya, Takaishi, and Kawazoe (2000). In general, antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups. The essential requirement for effective radical scavenging is the 3′,4′-orthodihydroxy configuration in ring B and 4-carbonyl group in ring C. The presence of 3-OH group or 3- and 5-OH groups, giving a catechol-like structure in ring C, is also beneficial for the antioxidant activity of flavonoids. The presence of the C2–C3 double bond configured with a 4-keto arrangement is known to be responsible for electron delocalisation from ring B and it increases the radical-scavenging activity. In the absence of the *o*-dihydroxy structure in ring B, a catechol structure in ring A can compensate for flavonoid antioxidant activity. Rutin has a catechol structure in ring B, as well as a 2, 3-double bond in conjunction with a 4-carbonyl group in ring C, allowing for delocalisation of the phenoxyl radical electron to the flavonoid nucleus. Flavonols have a hydroxyl group at position 3, which suggests a structurally important role of the 3-OH group of the chroman ring responsible for enhancement of antioxidant activity. The unsaturation in the C ring of flavonoids allows electron delocalisation across the molecule for stabilisation of the aryloxyl radical (Rastija & Medić-Šarić, in press). Among the flavonoids identified in our study, 3′,6-dimethoxyapigenin has two methoxy in rings A and B and appears to be a weaker antioxidant than apigenin, without any methoxy substitution. Presence and the number of free OH groups is a determining factor in antioxidant activity. Rutin, apigenin and 3′,7-dimethoxyapigenin with three free OH groups, exhibited more antioxidant activity than 4′,6-dimethoxyapigenin. Lipid peroxidation is one of the major causes of quality deterioration in lipid-containing foods. It affects the colour, flavour, texture, and nutritive value of foods. However, the use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds. This situation has created a necessity for scrutinizing naturally occurring antioxidant substances which may be used in foods in place of synthetic antioxidants. Plant phenolics can delay the onset of lipid oxidation and decomposition of hydroperoxides in food products as well as in living tissues. These antioxidants can be concentrated either as crude extracts or individual phenolic compounds to be used in food products especially highly unsaturated oils (Wettasinghe & Shahidi, 1999). Flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties. Apart from the fat-soluble tocopherols, the most common and active antioxidant compounds naturally occurring in foods are the flavonoids, possessing activity in both the hydrophilic and lipophilic systems.

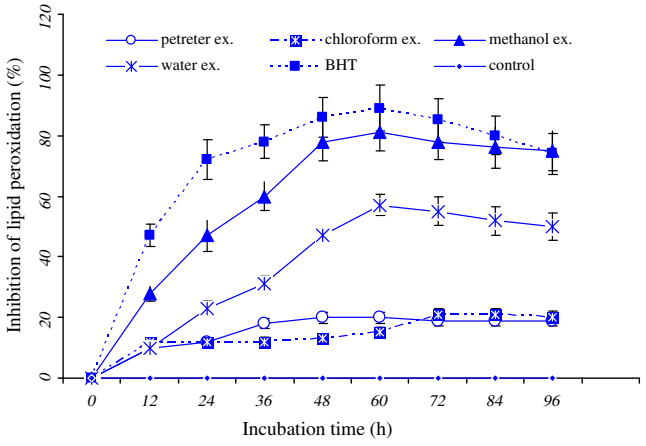

Fig. 1. Antioxidant activity of various extracts from *T. polium* by ATC method.

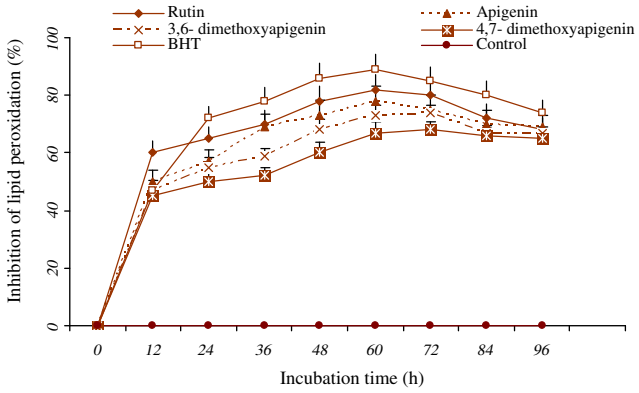

Fig. 2. Antioxidant activity of isolated flavonoids from *methanol extract of T. polium* by ATC method.

It is widely believed that the antioxidant ability of flavonoids resides mainly in their ability to donate hydrogen atoms and thereby scavenge the free radicals generated during lipid peroxidation (Amić, Davidović-Amić, Bešlo, & Trinajstić, 2003). Therefore dietary intake of flavonoid-containing foods was suggested to be of benefit as free radical preservatives. By regarding the potentially antioxidant activity and rich content of flavonoids of *T. polium*, this plant may be added to various food products in place of synthetic antioxidants in order to retard lipid peroxidation. These results here also show that in general, the rich-flavonoids plants, could be a good source of antioxidants that would help to increase the overall antioxidant capacity of an organism and protect it against lipid peroxidation. At last, to better manage clinical consequences arising from oxidative damage, detailed information on the structure of the most active compounds of the plant must be investigated and other flavonoids to be identified and further biological tests should be conducted. That work will be the core of the next scientific communications.

Acknowledgements

The present article is a part of a research project which has been supported by the Kerman University of Medical science, Vice Chancellor for Research. The authors are grateful for financial support of this work.

References

Abdollahi, M., Karimpour, H., & Monsef-Esfehani, H. R. (2003). Anti-nociceptive effects of *Teucrium polium* L total extract and essential oil in mouse writhing test. *Pharmacological Research, 48*(1), 31–35.

Amić, D., Davidović-Amić, D., Bešlo, D., & Trinajstić, N. (2003). Structure-radical scavenging activity relationship of flavonoids. *Crotaica Chemica Acta, 76*(1), 55–61.

Babenko, N. A., & Shakhova, E. G. (2006). Effects of *Chamomilla recutita* flavonoids on age-related liver sphingolipid turnover in rats. *Experimental Gerontology, 41*(1), 32–39.

Bedir, E., Tasdemi, D., Çalis, I., Zerbe, O., & Sticher, O. (1999). Neo-clerodane diterpenoids from *Teucrium polium*. *Phytochemistry, 51*, 921–925.

Bondet, V., Williams, W. B., & Berset, C. (1997). Kinetic and mechanism of antioxidant activity using the DPPH free radical method. *Lebensmittel-Wissenschaft Un Technologie, 30*, 609–615.

Burda, S., & Oleszek, W. (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agriculture and Food Chemistry, 49*, 2774–2779.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*(5), 323–328.

del Baño, M. J., Lorente, J., Castillo, J., Benavente-García, O., Antonio del Río, J., Ortuno, A., Quirin, K. W., & Gerard, D. (2003). Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis* antioxidant activity. *Journal of Agricultural and Food Chemistry, 51*(15), 4247–4253.

Esmaeili, A. M., & Yazdanparast, R. (2004). Hypoglycaemic effects of *Teucrium Polium*: Studies with rat pancreatic islets. *Journal of Ethnopharmacology, 95*(1), 27–30.

Facciola, S. (1990). *Cornucopia – A source book of edible plants*. Kampong Publications.

Graven, E. H., Deans, S. G., Svoboda, K. P., Mavi, S., & Gundidza, M. G. (1992). Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq. *Flavour and Fragrance Journal, 7*(3), 121–123.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure–activity relationship. *The Journal of Nutritional Biochemistry, 13*(10), 572–584.

Hu, C., & Kitts, D. D. (2005). Dendelion (*Taraxacum officinale*) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. *Phytomedicine, 12*(8), 588–597.

Katalinic, V., Milos, M., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94*(4), 550–557.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technology, 47*, 85–89.

Ljubuncic, P., Dakwar, S., Portnaya, I., Cogan, U., Azaizeh, H., & Bomzon, A. (2006). Aqueous extracts of *Teucrium polium* possess remarkable antioxidant activity *in vitro*. *Evidence Based Complementary Alternative Medicine, 3*(3), 329–338.

Masude, T., Isibe, D., Jitoe, A., & Naramati, N. (1992). Antioxidant curcuminoids from rhizomes of *Curcuma zanthorrhiza*. *Phytochemistry, 33*, 3645–3647.

Mozaffarian, V. (1997). *Dictionary of the names of Iranian plants*. Tehran, Iran: Frahang Mo'aser Pub.

Proestos, C., Sereli, D., & Komaitis, M. (2006). Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. *Food Chemistry, 95*(1), 44–52.

Rasekh, H. R., Khoshnood-Mansourkhani, M. J., & Kamalinejad, M. (2001). Hypolipidemic effects of *Teucrium polium* in rats. *Fitoterapia, 72*(8), 937–939.

Rastija, V., & Medić-Šarić, M. (in press). QSAR study of antioxidant activity of wine polyphenols. *European Journal of Medicinal Chemistry*.

Shahidi, F. (1997). *Natural antioxidants: Chemistry, health effects, and applications*. Urbana, IL: AOCS Press.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Review in Food Science and Nutrition, 32*, 67–103.

Silva, B. A., Ferreres, F., Malva, J. O., & Dias, A. C. P. (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chemistry, 90*(1–2), 157–167.

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. *Food Chemistry, 101*(3), 1012–1018.

Tariq, M., Ageel, A. M., Al-Yahya, M. A., Mossa, J. S., & Al-Said, M. S. (1989). Anti-inflammatory activity of *Teucrium polium*. *International Journal of Tissue Reactions, 11*(4), 185–188.

Wettasinghe, M., & Shahidi, F. (1999). Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. *Food Chemistry, 67*(4), 399–414.

Williams, G. M., & Jeffrey, A. M. (2000). Oxidative DNA damage: Endogenous and chemically induced. *Regulatory Toxicology and Pharmacology, 32*(3), 283–292.

Yang, J., Guo, J., & Yuan, J. (2008). In vitro antioxidant properties of rutin. *LWT – Food Science and Technology, 41*(6), 1060–1066.

Ye-ilada, E., Tsuchiya, K., Takaishi, Y., & Kawazoe, K. (2000). Isolation and characterization of free radical scavenging flavonoid glycosides from the flowers of *Spartium junceum* by activity-guided fractionation. *Journal of Ethnopharmacology, 23*(3), 471–478.