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Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*

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Abstract

Total phenolic content, concentration of flavonoids and in vitro antioxidant activity of twenty different extracts from the whole plant and plant parts (leaves, flowers and stems) of *Teucrium polium* were determined. The total phenolic contents ranged between 14.57 to 157.84 mg of GaA/g of extract. The concentrations of flavonoids varied from 6.48 to 139.87 mg of Ru/g of extract. Antioxidant activity was determined in vitro using DPPH reagent and expressed as concentration of each extract required to inhibit radical by 50% (IC_{50}) values that ranged from 26.30 to 2190.75 µg/ml. The methanolic leaves extract contain the greatest concentration of phenolic compounds (157.84 mg of GaA/g) and showed strong antioxidant activity (IC_{50} = 26.30 µg/ml). Ginkgo and Green tea extracts were analyzed for comparison, and the results indicated that some extracts of *T. polium* were equal in activity with Ginkgo or Green tea and some appeared to have greater activity. The obtained results suggest strong antioxidant activity and large contribution of separate analysis for the maximum exploitation of active phenolic compounds from *T. polium*. Based on this information, plant parts of this plant are natural sources of antioxidant substances of high importance.

Keywords: *Teucrium polium* subsp. *polium*, antioxidant activity, phenolics, flavonoids

Introduction

Recently, there is growing interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [1]. Antioxidants are secondary constituents or metabolites found naturally in plants. They are mainly phenolics serving in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Their antioxidant activity is related to their redox properties, ability to scavenge a variety of reactive species such as superoxide, hydroxyl and peroxyl radicals and hypochlorous acid, singlet oxygen quenching, metal ion chelation [2,3]. It was reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds [4].

Teucrium polium L. subsp. *polium* (Felty germander) – belongs to the family Lamiaceae Lindely, subfamily *Ajugoideae* Kostel and section *Polium* (Miller) Schreber. *T. polium* is a wild-growing perennial herbaceous flowering plant with little branched stems up to 40 cm high. Leaves are without or with very short petiole, oval-elongated and slightly incised. Flowers are white to light pink, densely clustered at the top of the branch. Inhabit rocky limestones and dry mountain meadows in the Mediterranean region and Middle East [5]. *T. polium* is best popular species of this genus in the folk medicine, very often used for tea and tonic preparing during treatment of appetite loss, gastrointestinal ailments and as a spice plant. Numerous in vitro and in vivo tests of biological activity prove antibacterial, antifungal, antiinflammatory, antiproliferative, apoptotic, antinociceptive, antihypertensive, hepatoprotective and hypoglycemic properties [6]. Extracts from *T. polium* potentiates the cytotoxic and apoptotic effects of anticancer drugs: vincristine, vinblastine and doxorubicin, against a panel of cancerous cell lines [7].

The therapeutic benefits of *T. polium* extracts are usually attributed to their ability to suppress oxidative processes [8]. Numerous investigations showed the antioxidant activity of *T. polium* extracts, it was reported that the alcoholic extract of *T. polium* had a suppressing effect on hydrogen peroxide-induced lipid peroxidation in red blood cells [9], the ability of water extract to suppress Fe^{2+}-induced lipid peroxidation in rat liver homogenates [10].

The objective of this study was to examine the distribution of phenolic compounds, as the carriers of antioxidant activity, in different vegetative parts of the plant, and what are the most suitable solvents for the production of extracts that are rich in phenolic compounds from *T. polium*. Separate examination of plant parts allows a significant contribution to medicinal plant study and their pharmaceutical applications [11]. Twenty

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extracts were obtained from *T. polium* using different solvents for extraction of whole plant and its vegetative parts, separately. For each extract total phenolic content and flavonoid concentrations were determined using spectrophotometric methods. 2,2-dyphenyl-1-picrylhydrazyl (DPPH) scavenging activity of each extract was also determined and compared to reference natural and synthetic antioxidants [chlorogenic acid, rutin and 3-tert-butyl-4-hydroxyanisole (BHA)]. The obtained results were also compared with the values of standard synthetic antioxidants. A parallel analysis of Ginkgo (*Ginkgo biloba* L.) and Green tea [*Camellia sinensis* (L.) Kuntze] as most popular plants rich in natural antioxidants was carried out and compared with the values related to *T. polium*.

Material and methods

Plant material

In August 2009 aerial flowering parts of *T. polium* were collected from natural populations in the region of Suva Planina Mt. in Southeast Serbia: (position: 43°19′14.82″ N, 22°10′21.42″ E, altitude: 302.00 m, exposition: W, substratum: limestone). The voucher specimen of *T. polium* L. 1753. subsp. *polium* No. 2-2212, UTM 34 TEN 98, August 9th, 2009, det: Milan Stanković; rev: Goran Anačkov, were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Natural Science, University of Novi Sad. The collected plant material was air-dried in darkness at ambient temperature (20°C). The dried material was cut up and stored in tightly sealed dark containers until needed.

Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from "Zorka pharma" Šabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid and DPPH were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, butylated hydroxyanisole (BHA) and aluminium chloride hexahydrate ($AlCl_3$) were purchased from Fluka Chemie AG, Buchs, Switzerland. All other solvents and chemicals were of analytical grade. The samples of Green tea (*Camellia sinensis*) were purchased from a local pharmacy. A standardized extract of *Ginkgo biloba* was obtained from Pharmaceutical Company "Ivančić i Sinovi", Belgrade, Serbia (base for dietary products *Ginkgo biloba* extract, produced by Sichuan Xieli Pharmaceutical. Co. Ltd., Sichuan, China).

Preparation of plant extracts

Prepared plant material (10 g) was transferred to dark-coloured flasks and with 200 ml of solvent (water, methanol, ethyl acetate, acetone, petroleum ether) respectively and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40°C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4°C.

Determination of total phenolic contents in the plant extracts

The total phenolic content was determined using spectrophotometric method [12]. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The samples were incubated at 45°C for 15 min. The absorbance was determined at λ_{max} = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution.The same procedure was repeated for the gallic acid and the calibration line was construed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GaA/g of extract).

Determination of flavonoid concentrations in the plant extracts

The concentrations of flavonoids was determined using spectrophotometric method [13]. The sample contained 1 ml of methanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined at λ_{max} = 415 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the rutin and the calibration line was construed. Concentration of flavonoids in extracts was expressed in terms of rutin equivalent (mg of Ru/g of extract).

Evaluation of DPPH scavenging activity

The ability of the plant extract and reference substance to scavenge DPPH free radicals was assessed using the method described by Tekao et al. [14], adopted with suitable modifications from Kumarasamy et al. [15], The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 μg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH methanolic solution (80 μg/ml). After 30 min in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using equation: % inhibition = 100 × (A of control − A of sample)/A of control, whilst concentrations of extracts required to inhibit radical by 50% (IC_{50}) values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis. The data were presented as mean values ± standard deviation (N = 3).

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation. The magnitude of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. XII, 2004).

Results and discussion

The results of the total phenolic content determination of the twenty different extracts from whole plant, leaves, flowers and stems of *T. polium*, obtained using five different solvents (water, methanol, ethyl acetate, acetone and petroleum ether), are presented in Fig. 1. The content of total phenolic compounds in extracts, expressed as gallic acid equivalents per gram of dry extract, ranged between 14.57 to 157.84 mg of GaA/g. The result in the Fig. 1 indicates a significant difference between concentrations of phenolics in different plant parts. Total phenolic content was the highest in methanolic extracts

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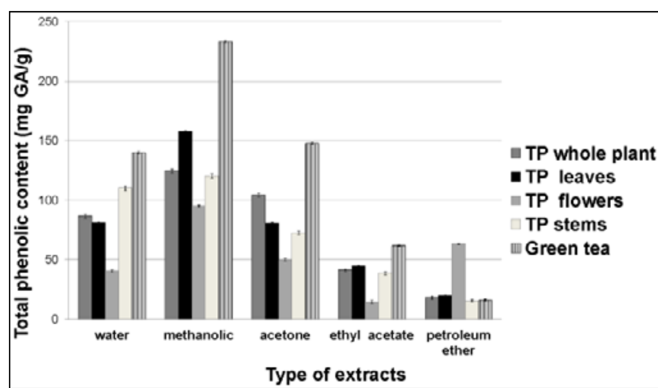

Fig. 1 Total phenolic contents in the plant extracts of *T. polium* expressed in terms of gallic acid equivalent (mg of GaA/g of extract).

from different parts of *T. polium*, among which methanol extract of leaves (157.84 mg of GaA/g) contained the highest concentracion of phenolic compound. Water and acetone extracts also contained high levels of phenolics, while contents of phenolics in ethyl acetate and petroleum ether extracts was lower. The results revealed that total phenolic contents in flowers extracts were significantly lower than those obtained from other plant parts, with the exception of petroleum ether extracts. The petroleum ether extract of flowers had higher total phenolic content (63.45 mg of GaA/g) than whole plant, leave and stem extracts obtained from the same solvent. Phenolic content for Green tea samples analyzed in the study ranged from 16.02 to 233.68 mg of GaA/g dry extract (Tab. 1). Among the analyzed extracts, methanolic extract of Green tea had the highest phenolic content (233.68 mg/g) followed by acetone (147.77 mg/g) and water (140.11 mg/g) extracts. The petrol ether extracts of whole plant, leaves and flovers of *T. polium* had a higher concentrations of phenolic compounds in relation to the petrol ether extract of Green tea. The extract from other solvents of *T. polium* had significantly lower concentration of phenolic compounds than those obtained from Green tea. Phenolic content of *G. biloba* standardized extract was 140.18 mg of GaA/g dry sample (Tab. 2). Methanol extract of *T. polium* leaves (157.84 mg of GaA/g) had a higher concentration of phenolic compounds than *G. biloba* standardized extract, while the levels of phenolic compounds in other extracts of *T. polium* were less.

The variability in the total phenolic contents in different extracts could be the result of the varying solubility of the phenolic compounds; this variation in solubility may be driven by the solvent polarity [16]. Some studies showed that methanol and ethanol were better extraction solvents for phenolics from plant materials than less polar solvents including acetone and hexane [17,18]. The solubility of phenolics is governed by the chemical nature of the plant sample, as well as the polarity of the solvents used. Plant materials may contain phenolics varying from simple (e.g., phenolic acids, anthocyanins) to highly polymerized substances (e.g., tannins) in different quantities. Moreover, phenolics may also be associated with other plant components such as carbohydrates and proteins. Therefore, there is no universal extraction procedure suitable for extraction of all plant phenolics. Solvents, such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water [19]. According to another study, a less polar solvent such as acetone could extract more phenolic compounds from the flowers than more polar solvents, including methanol and water. These differences may be due to the types of phenolic compounds in the plant materials. In general, a good balance in polarity is needed in extracting phenolics from plant sources [20,21]. In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone. Ethanol is another good solvent for polyphenol extraction and is safe for human consumption [22,23].

The concentration of flavonoids in various extracts of *T. polium* was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalents (mg of Ru/g of extract). The summary of quantities of flavonoids identified in the tested extracts is shown in Fig. 2. The concentrations of flavonoids in plant extracts ranged from 6.48 to 139.87 mg of Ru/g. The acetone extract of leaves showed higher amounts of flavonoid content (139.87 mg of Ru/g) than the other examined extracts. In contrast, flavonoid concentrations of water and petroleum ether extracts were the lowest. The obtained values of the flavonoid concentrations in Green tea varied from 34.18 to 335.40 mg of Ru/g (Tab. 1). In the comparison of flavonoid concentrations between Green tea and *T. polium* only the methanolic extracts of *T. polium* showed higher or approximately equal values than methanolic extract of Green tea. Water, acetone, ethyl acetate and petroleum ether extracts of Green tea had greater concentration of flavonoids than *T. polium* extracts obtained from the same solvents. Based on the flavonoid contents of the extracts, acetone was the best extraction solvent to extract flavonoids from *T. polium* and Green tea. The flavonoid concentration in *G. biloba* standardized extract was 192.69 mg of Ru/g, (Tab. 2) – higher than the values of all *T. polium* extracts. Based on the flavonoid contents of the extract, acetone was the best extraction solvent to extract flavonoids from *T. polium* and Green tea.

The concentration of each extract required to inhibit radical by 50% (IC_{50}) is shown in Tab. 3. Parallel to the examination

Tab. 1 Antioxidant (DPPH scavenging) activity of investigated plant extracts from *T. polium* presented as IC_{50} values (μg/ml).

Type of analisys	Water	Methanolic	Acetone	Ethyl acetate	Petroleum ether
total phenolic content	140.11 ±0.29	233.68 ±0.18	147.77 ±0.77	61.94 ±0.19	16.02 ±0.29
concentrations of flavonoids	195.41 ±1.04	47.80 ±0.44	335.40 ±0.71	242.95 ±1.45	34.18 ±0.85
antioxidant activity	20.62 ±1.06	14.50 ±1.69	28.59 ±1.12	61.43 ±1.16	238.25 ±2.01

Each value is the average of three analyses ± standard deviation.

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Tab. 2 Values of antioxidant (DPPH scavenging) activity of standard substances obtained for comparison with the values of *T. polium*.

Type of analysis	Values
total phenolic content	140.18 ±0.26
concentration of flavonoids	192.69 ±0.72
antioxidant activity	33.91 ±1.16

Each value is the average of three analyses ± standard deviation.

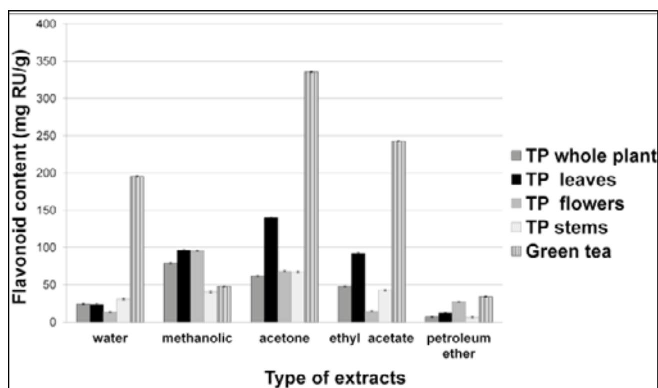

Fig. 2 Flavonoid concentrations in the plant extracts of *T. polium* expressed in terms of rutin equivalent (mg of Ru/g of extract).

of the antioxidant activity of the plant extracts, the values for two well-known medicinal plants – Green tea (Tab. 1) and *G. biloba* (Tab. 2) – were obtained and compared to the values of the antioxidant activity. The reference antioxidants used were rutin, chlorogenic acid and BHA. The scavenging activity of the reference compounds are summarized in Tab. 4. In comparison to antioxidant activity of pure reference antioxidants (Tab. 4), investigated under same conditions, had IC_{50} value between 11.65 and 5.39 μg/ml, hence, the activities of the extracts were moderate. The investigated extracts of *T. polium* demonstrated very different radical-scavenging activities and IC_{50} values varied between 2190.75 and 26.30 μg/ml. The methanolic leaves extract showed the greatest potency (IC_{50} = 26.30 μg/ml) among the twenty extracts, and exhibited the greatest radical-scavenging activity. When compared to the activity of natural and synthetic standards, such as rutin (IC_{50} = 9.28 μg/ml), chlorogenic acid (IC_{50} = 11.65 μg/ml) and BHA (IC_{50} = 5.39 μg/ml), DPPH scavenging activity of methanolic leaves extract is very high, considering that the extract is a mixture of a great number of components opposite pure compounds used as standards. Methanolic extract of flowers also showed considerable DPPH scavenging activity with IC_{50} value of 41.23 μg/ml. Water (IC_{50} = 56.40 μg/ml) and methanol (IC_{50} = 59.37 μg/ml) extracts of whole plant, as well as water (IC_{50} = 59.12 μg/ml) and methanol (IC_{50} = 59.28 μg/ml) extracts of stems showed similar antioxidant capacity, while acetone extracts had less DPPH scavenging activity. In average, the petroleum ether and ethyl acetate extracts were the least active in comparison of the corresponding extracts obtained from other solvents.

The values of antioxidant activity of Green tea obtained for comparison with *T. polium* ranged from 14.50 to 238.25 μg/mL. Results (Tab. 4) clearly indicate that all extracts exhibited antioxidant activity as follows: methanol > water > acetone > ethyl acetate > petroleum ether. Comparing the antioxidant activity of Green tea and *T. polium*, only the methanol extract from leaves of *T. polium* showed values approximate to Green tea, while the rest of the extracts were with less activity than the corresponding extracts of Green tea. The value of antioxidant activity of *G. biloba* standardized extract was 33.91 μg/ml, (Tab. 2). In comparison, methanol extract from leaves of *T. polium* showed higher activity than *G. biloba*, while methanolic extract of flowers *T. polium*, showed particularly high free radical scavenging activity (IC_{50} = 41.23 μg/ml), close to the activity of *G. biloba* standardized extract. All the other extracts of *T. polium* showed smaller activity than *G. biloba* extracts.

In the previous phytochemical screening of *T. polium* phenylethanoid glycosides (verbascoside and poliumoside), apigenin, 4',7 dimethoxy apigenin, 3',6 dimethoxy apigenin and rutin were identified as very active compounds. For flavonoids found in a *T. polium* in a number of studies demonstrated the significant antioxidant activity [24]. Phenolic compounds possess ideal structure chemistry for free radical scavenging activities because they have phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical and extended conjugated aromatic system to delocalize an unpaired electron. As an alternative antioxidant property, some phenolic compounds with dihydroxy groups can conjugate transition metals, preventing metal-induced free radical formation [19]. Comparing the concentration of phenolic compounds and values for DPPH scavenging activity we found that extracts with the highest concentrations of phenolic compounds also have strong scavenging effect. Based on these results, each extracts of *T. polium* exhibited phenolic concentration-dependent scavenging effects. Numerous investigations of the antioxidant activity of plant extracts have confirmed a high

Tab. 3 Values of total phenolic content (mg of GaA/g of extract), flavonoid concentrations (mg of Ru/g of extract) and antioxidant activity (IC_{50} – μg/ml) of Green tea extracts obtained for comparison with the values of *T. polium*.

Type of extract	Water	Methanolic	Acetone	Ethyl acetate	Petroleum ether
whole plant	56.40 ±1.53	59.37 ±0.76	63.30 ±1.33	622.96 ±3.63	>1000
leaves	80.87 ±1.32	26.30 ±0.92	119.73 ±2.15	364.23 ±2.58	>1000
flowers	199.24 ±1.91	41.23 ±1.12	351.50 ±2.38	>1000	>1000
stems	59.12 ±1.26	59.28 ±1.01	138.64 ±2.01	475.58 ±2.96	2190.75 ±3.95

Each value is the average of three analyses ± standard deviation.

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Tab. 4 Values of total phenolic content (mg of GaA/g of extract) and flavonoid concentrations (mg of Ru/g of extract) and antioxidant activity (IC_{50} – µg/ml) of *G. biloba* standardized extract obtained for comparison with the values of *T. polium*.

Substances	IC_{50} µg/ml
BHA	5.39 ±0.31
rutin	9.28 ±0.27
chlorogenic acid	11.65 ±0.52

Each value is the average of three analyses ± standard deviation.

linear correlation between the values of phenolic content and antioxidant activity [25]. The difference in morpho-anatomical structure and physiological activity of different plant parts contribute to varying phenolic content and antioxidative activity values. The authors who have comparatively analyzed the antioxidant activity of different parts of other plants have obtained similar results. Our data confirms the results of Özen et al. [26], in their comparative study of *Urtica pilulifera*: leaf extracts have higher concentrations of phenolic compounds and greater antioxidant activity than other plant parts.

In addition, the phenolic contents of extracts depend on the plant part used in the experiment and solvents used for extraction, and not only the concentration of phenolic contents but also properties of these compounds contribute to the activites of different extracts. The obtained results indicate that the methanolic extract from the leaves of *T. polium* shows a stronger antioxidant activity than those from the other plant parts also equal in activity with Ginkgo or Green tea. It seems the reason for this difference just is that the phenolic content in the methanolic extract from the leaves is higher than in those from other parts. Comparative analysis of different plant parts can be helpful when estimating the beneficial properties of *T. polium* extracts as valuable medicinal raw plant materials to be used for natural antioxidants in phytopharmacy. Further studies of this plant species should be directed to a detailed qualitative analysis of all its parts and carry out in vivo evaluation antioxidant of antioxidant properties.

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References

1. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem. 2001;49(11):5165-5170. http://dx.doi.org/10.1021/jf010697n
2. Halliwell B, Rafter J, Jenner A. Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? Am J Clin Nutr. 2005;81(1):268S-276S.
3. Rohma A, Riyanto S, Yuniarti N, Saputra WR, Utami R, Mulatsih W. Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (*Pandanus conoideus* Lam). Int Food Res J. 2010;17:97-106.
4. Moein S, Moein RM. Relationship between antioxidant properties and phenolics in *Zhumeria majdae*. J Med Plants Res. 2010;4(7):517-521.
5. Diklić N. Teucrium. In: Josifović M, editor. Flore de la Republique Socialiste de Serbie. Belgrade: Serbian Academy of Sciences and Arts; 1974. p. 349-356.
6. Jurisić R, Vladimir-Knezević S, Kalodera Z, Grgić J. Determination of selenium in Teucrium species by hydride generation atomic absorption spectrometry. Z Naturforsch C J Biosci. 2003;58(1-2):143-145.
7. Rajabalian S. Methanolic extract of *Teucrium polium* L. potentiates the cytotoxic and apoptotic effects of anticancer drugs of vincristine, vinblastine and doxorubicin against a panel of cancerous cell lines. Exp Oncol. 2008;30(2):133-138.
8. Shtukmaster S, Ljubuncic P, Bomzon A. The effect of an aqueous extract of *Teucrium polium* on glutathione homeostasis in vitro: a possible mechanism of its hepatoprotectant action. Adv Pharmacol Sci. 2010;2010:1-7. http://dx.doi.org/10.1155/2010/938324
9. 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(4):280-284. http://dx.doi.org/10.1002/ptr.1380
10. 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(1):43-47. http://dx.doi.org/10.1016/j.jep.2005.01.060
11. Siddique AN, Mujeeb M, Najmi AK, Akram M. Evaluation of antioxidant activity, quantitative estimation of phenols and flavonoids in different parts of *Aegle marmelos*. Afr J Plant Sci. 2010;4(1):1-5.
12. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth Enzymol. 1999;299:152-178. http://dx.doi.org/10.1016/S0076-6879(99)99017-1
13. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, et al. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol. 2000;72(1-2):35-42. http://dx.doi.org/10.1016/S0378-8741(00)00196-3
14. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem. 1994;58(10):1780-1783. http://dx.doi.org/10.1271/bbb.58.1780
15. Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother Res. 2007;21(7):615-621. http://dx.doi.org/10.1002/ptr.2129
16. Marinova EM, Yanishlieva NV. Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chem. 1997;58(3):245-248. http://dx.doi.org/10.1016/S0308-8146(96)00223-3
17. Zarena AS, Sankar KU. A study of antioxidant properties

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from *Garcinia mangostana* L. pericarp extract. Acta Sci Pol Technol Aliment. 2009;8:23-34.

18. Mohsen SM, Ammar ASM. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 2009;112(3):595-598. http://dx.doi.org/10.1016/j.foodchem.2008.06.014
19. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313-7352. http://dx.doi.org/10.3390/molecules15107313
20. Liu S, Lin J, Wang C, Chen H, Yang D. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. Food Chem. 2009;114(2):577-581. http://dx.doi.org/10.1016/j.foodchem.2008.09.088
21. Horax R, Hettiarachchy N, Chen P. Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of bitter melons (*Momordica charantia*) harvested at three maturity stages (immature, mature, and ripe). J Agric Food Chem. 2010;58(7):4428-4433. http://dx.doi.org/10.1021/jf9029578
22. Metivier RP, Francis FJ, Clydesdale FM. Solvent extraction of anthocyanins from wine pomace. J Food Sci. 1980;45(4):1099-1100. http://dx.doi.org/10.1111/j.1365-2621.1980.tb07534.x
23. Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y. Extraction of polyphenolics from plant material for functional foods-engineering and technology. Food Rev Int. 2005;21(1):139-166. http://dx.doi.org/10.1081/FRI-200040606
24. Silva MM, Santos MR, Caroço G, Rocha R, Justino G, Mira L. Structure-antioxidant activity relationships of flavonoids: a re-examination. Free Radic Res. 2002;36(11):1219-1227. http://dx.doi.org/10.1080/198-1071576021000016472
25. Leccese A, Viti R, Bartolini S. The effect of solvent extraction on antioxidant properties of apricot fruit. Cent Eur J Biol. 2011;6(2):199-204. http://dx.doi.org/10.2478/s11535-010-0113-2
26. Özen T, Çöllü Z, Korkmaz H. Antioxidant properties of *Urtica pilulifera* root, seed, flower, and leaf extract. J Med Food. 2010;13(5):1224-1231. http://dx.doi.org/10.1089/jmf.2009.1303

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