ARTICLE DOI: 10.5504/BBEQ.2011.0020 A&EB

TOTAL PHENOLIC CONTENT, FLAVONOID CONCENTRATIONS AND ANTIOXIDANT ACTIVITY, OF THE WHOLE PLANT AND PLANT PARTS EXTRACTS FROM *TEUCRIUM MONTANUM* L. VAR. *MONTANUM*, F. *SUPINUM* (L.) REICHENB.

Milan S. Stankovic[1], Neda Niciforovic[2], Marina Topuzovic[1], Slavica Solujic[2]
[1]University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Kragujevac, Republic of Serbia
[2]University of Kragujevac, Faculty of Science, Department of Chemistry, Kragujevac, Republic of Serbia
Correspondence to: Milan S. Stankovic
E-mail: mstankovic@kg.ac.rs

ABSTRACT

The aim of this study was to determine the total phenolic content, concentrations of flavonoids and in vitro antioxidant activity of twenty different extracts from the plant and plant parts (leaves, flowers and stems) of Teucrium montanum L. var. montanum, f. supinum (L.) Reichenb. The total phenolic content in the extracts was determined using Folin-Ciocalteu reagent and their amounts ranged between 8.33 to 169.06 mg GA/g. The concentrations of flavonoids in plant extracts varied from 3.96 to 88.31 mg RU/g. Antioxidant activity was analyzed in vitro using DPPH reagent, activity expressed as IC_{50} and obtained results ranged from 29.41 to 2408.47 µg/mL. Parallel to the analysis of T. montanum, Ginkgo biloba L. and Green tea (Camellia sinensis (L.) Kuntze) extracts were analyzed for comparison, and the results indicated that some extracts of T. montanum were equal in activity with Ginkgo or Green tea and some appeared to have greater activity. Based on these results of investigation, it could be concluded that T. montanum is a rich source of phenolic compounds as natural antioxidans of high value.

Biotechnol. & Biotechnol. Eq. 2011, **25**(1), 2222-2227

Keywords: *Teucrium montanum* L., Germander, phenolic content, flavonoids, antioxidant activity

Introduction

Mountain germander, *Teucrium montanum* L. var. *montanum*, f. *supinum* (L.) Reichenb. belongs to the family *Lamiaceae* Lindely, subfamily *Ajugoideae* Kostel and section *Polium* (Miller) Schreber. This is a perennial, shrub-like plant with half-ligneous branches, up to 25 cm high and inhabits thermophilic calcareous rocks, dry mountain meadows and edges of forests in Europe and Anatoly (8, 32). Medicinal plant species of the genus *Teucrium* are very rich in phenolic compounds with very strong biological activity and often used in ethnobotany medicine and pharmacy. The most popular species of this genus in the flora of Europe is *T. montanum* that is used as a diuretic and in the treatment of digestive and respiratory diseases and possesses antiinflammatory, antioxidative and antimicrobial effect (4, 9, 13, 20, 33).

Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems (21). Free radicals cause many human diseases like cancer, Alzheimer's disease, cardic reperfusion abnormalities, kidney and liver disease, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders and aging (7).

Many research studies have demonstrated that medicinal plants, fruits and vegetables contain various components with antioxidant activity, which are responsible for their beneficial health effects. In addition to vitamin C, vitamin E and carotenoids, polyphenols (a wide class of components including phenolic acids, catechins, flavonols and anthocyanins), have shown strong antioxidant capacity (10). Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones (19). Most frequently used synthetic antioxidants in food industry at high doses, such as BHA, exhibit genotoxic and carcinogenic effect, while for BHT it is proven to cause hemorraging (5, 11, 12).

Separate examination of plant parts allows a significant contribution to medicinal plant study and their pharmaceutical applications (15, 25). The basic aim of the presented research was to determine the contents of phenolics and concentrations of flavonoids in various extracts of the species *T. montanum* using spectrophotometric methods, as well as to examine the antioxidant activity of plant extracts *in vitro* using standard model system. In addition, we compared the results obtained from the whole plant extracts with the results of analysis of extracts from different plant parts such as leaves, flowers, and stems and assessed the significance of particular analysis for effective use in pharmacy. The obtained results of antioxidant activity were also compared with the values of reference 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. montanum*.

Materials and Methods

Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from „Zorka pharma“ Sabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid and 2,2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, 3-tert-butyl-4-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 „Ivancic i Sinovi“, Belgrade, Serbia (base for dietary products *Ginkgo biloba* extract, produced by Sichuan Xieli Pharmaceutical. Co. Ltd., Sichuan, China).

Plant material

In July 2009 aerial flowering parts of *T. montanum* were collected from natural populations in the region of Goc Mt. in Central Serbia: (position: 43° 36’ 43.56” N, 20° 41’ 52.25” E, altitude: 381.91 m, exposition: W, substratum: serpentinite). The voucher specimen of *T. montanum* L. 1753, No 2-2209, UTM 34 TDP 82, Jule 16th 2009., det.: Milan Stankovic; rev.: Goran Anackov, 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^{o}C). The dried plant material was cut up and stored in tightly sealed dark containers until needed.

Preparation of plant extracts

The air-dried plant material (10 g) was coarsely crushed in small pieces of 2-6 mm using the cylindrical crusher and extracted with organic solvents (water, methanol, acetone, ethyl acetate, petroleum ether). The extract was filtered through a paper filter (Whatman, No. 1) and evaporated under reduced pressure by the rotary evaporator. The obtained extracts (**Table 1**) were stored in dark glass bottles for further processing.

Determination of total phenolic content

The total soluble phenolic compounds in the different extracts of *T. montanum* were determined with Folin-Ciocalteu reagent using gallic acid as a standard (26). Extracts were diluted to the concentration of 1 mg/mL in methanol and 0.5 mL of the soluted extract was mixed with 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of $NaHCO_3$ (7.5%). After 15 min at 45°C, the absorbance was measured at 765 nm versus blank sample on spectrophotometer (ISKRA, MA9523-SPEKOL 211). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Determination of flavonoid content

The total flavonoid content was determined spectrophotometrically according to standard method (22). Briefly, 0.5 mL of 2% solution of $AlCl_3$ in ethanol was mixed with the same volume of extract (500 µg/mL). Absorption readings at 415 nm were taken after 1 h against a blank (methanol). The total flavonoid content was determined using a standard curve with rutin (0-50 mg/L). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

Evaluation of DPPH scavenging activity

The ability of the plant extract to scavenge 1,1-dyphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed by the method described by Tekao et al. (29), adopted with suitable modifications from Kumarasamy et al. (14). DPPH (20 mg) was dissolved in methanol (250 mL) to obtain the concentration of 80 µg/mL. 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 DPPH (1 mL). After 30 min in darkness at room temperature (23^{o}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 [1], whilst 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).

$$\%\ inhibition = \left(\frac{A\ \text{control} - A\ \text{sample}}{A\ \text{control}} \right) x\,100$$

Statistical analysis

All experimental measurements were carried out in triplicate and were 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. 17, 2008).

Results and Discussion

Total phenolic content

Total phenolic and flavonoid content, and antioxidant activity *in vitro* were determined for water, methanol, acetone, ethyl acetate and petroleum ether extracts of whole *T. montanum* plant as well as for leaves, flowers and stems, separately. Various solvents were used to achieve extraction of active substances with diversity in their polarity. This choice of solvents proved to be very effective in earlier studies (27). In this manner twenty different extracts were obtained. After extraction of 10 g of dried plant material, the largest volume of crude extract was obtained using polar solvents (**Table 1**).

The results of the total phenolic content determination of the examined plant extracts, using Folin-Ciocalteu method, are presented in **Fig. 1**. The content of total phenols in extracts, expressed as gallic acid equivalents (GA) per gram of dry extract, ranged between 8.33 to 169.06 mg GA/g.

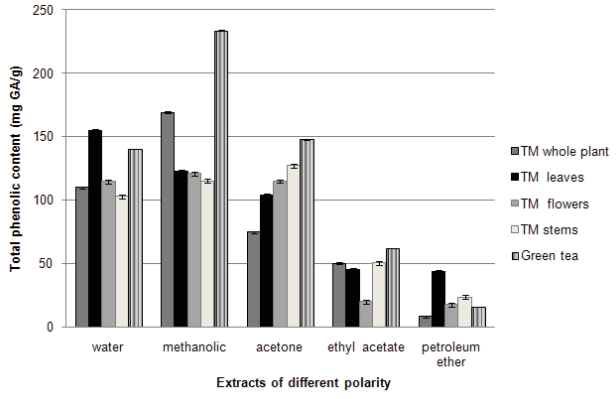

Fig. 1. Total phenolic contents in the plant extracts of *T. montanum* expressed in terms of gallic acid equivalent, GAE (mg of GA/g of extract)

In relation to the solvent used, high concentrations of phenolic compounds were found in water, methanolic and acetone extracts, among which methanol extract of whole plant (169.06 mg GA/g) and water extract of leaves (154.81 mg GA/g) contained the highest phenolic content. Ethyl acetate and petroleum ether extracts had low concentrations of phenolic compounds.

The concentrations of phenolic compounds in different parts of the plant, in relation to the concentration in whole plant extracts have unconformable values. In the group of water extracts phenol concentrations in the leaves is higher than the concentrations in the tested plant parts and whole plant extract. Between the tested methanolic extracts, in the extract of whole plant the highest concentration of phenolic compounds was measured in relation to the all of tested methanolic extracts. In other groups of extracts values are higher in plant parts in opposite to the value of whole plant extract.

In the comparison between the values of the plant parts different relationship can be found. In the extracts obtained using polar solvents (water and methanolic) order of concentrations for phenolic compounds is: leaves>flowers>stems. In acetone extracts from plant parts, order of concentrations is: stems>flowers>leaves, (**Fig. 1**).

Analyzing the results of total phenolic content in all extracts, it was noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity.

In the evaluation of new medicinal plants some authors compare their phytochemical characteristics with very well-known and widely used medicinal plants such as Green tea (6, 24). The values for total phenolic content of Green tea ranged from 16.02 to 233.68 mg GA/g (**Fig. 1**). Water extract of *T. montanum* (154.81 mg GA/g) has a higher concentration of phenolic compounds than the water extract of Green tea (140.11 mg GA/g). Also, petrol ether extracts of all plant parts and whole plant of *T. montanum* has a higher concentration of phenolic compounds in relation to the petrol ether extract of Green tea (**Fig. 1**).

The concentration of phenolic compounds in the extract of *G. biloba* was found to be 140.18 mg GA/g (**Table 4**). Water extract of leaves (154.81 mg GA/g) and methanol extract of whole plant (169.06 mg GA/g) have a higher concentration of phenolic compounds than the *G. biloba* extract.

The results of the authors, who analyzed the quantitative and qualitative phytochemical characteristics of plant organs, show different values for the leaf, stem and flower. Bystricka et al. (2), reported that concentration and dynamics of the polyphenol synthesis in plant organs depends on the plant species, type of organs and growth stage. The results of some authors who have comparatively analyzed the concentration

TABLE 1

The yields (g) of extracts of *T. montanum* obtained from 10 g dried plant material

Type of extract	water	methanolic	acetone	ethyl acetate	petroleum ether
whole plant	2.13	2.04	0.31	0.24	0.1
leaves	2.11	2.05	0.37	0.26	0.06
flowers	1.95	1.23	0.24	0.19	0.04
stems	1.68	1.04	0.4	0.09	0.16

TABLE 2

Antioxidant (DPPH scavenging) activity of investigated plant extracts of *T. montanum* presented as IC_{50} values[1] (μg/mL)

Type of extract	water	methanolic	acetone	ethyl acetate	petroleum ether
whole plant	29.41±0.76	45.41±0.85	108.10±1.09	220.98±2.64	1388.03±9.02
leaves	47.78±1.67	50.14±1.01	77.94±1.14	272.19±2.91	400.28±5.12
flowers	38.33±1.02	42.40±1.03	51.06±0.56	617.45±1.14	2408.47±8.39
stems	34.30±0.98	71.14±1.54	54.99±0.91	211.15±4.33	858.81±7.54
Green tea	20.62±1.06	14.50±1.69	28.59±1.12	61.43±1.16	238.25±2.01

[1]Each value in the table was obtained by calculating the average of three analyses ± standard deviation

of phenolic compounds in plant parts, support the fact that the highest concentration of phenolic compounds is found in leaves. In the analysis of Arash et al. (1), methanolic extracts of different organs from *A. paniculata*, had higher concentration of phenolic compounds in leaves extracts compared to the stem extracts, which is the same in relation to our results for the methanolic extract (**Fig. 1**).

Flavonoid concentrations

Because of their common presence in plants, flavonoids are important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative (28).

The concentration of flavonoids in various extracts of *T. montanum* was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of rutin equivalents (mg of RU per gram of dry 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 3.96 to 88.31 mg RU/g.

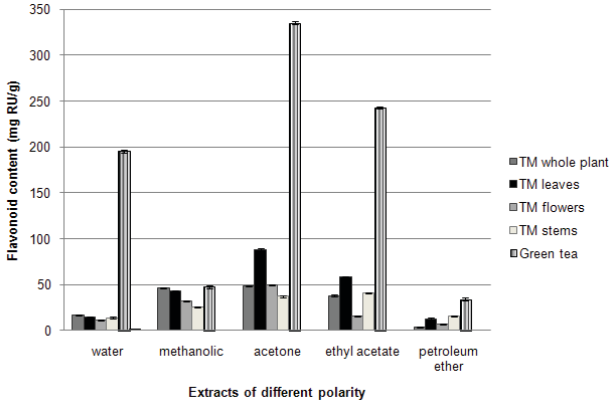

Fig. 2. Flavonoid concentrations in the different plant extracts of *T. montanum* expressed in terms of rutin equivalent (mg of RU/g of extract)

High concentrations of flavonoids were measured in acetone, and some ethyl acetate and methanolic extracts. Far the highest flavonoid content was found in acetone extract from the leaves of *T. montanum* (88.31 mg RU/g), followed by the leaf ethyl acetate extract with 58.48 mg RU/g. The lowest flavonoid concentration was measured in water and petroleum ether extracts. The concentration of flavonoids in various parts of the plant differed greatly from the value obtained for the whole plant (**Fig. 2**). These data are in agreement with other studies reporting flavonoid concentration in different plant parts (25).

The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (17). Based on the obtained values of the concentration of flavonoids in the examined extracts of *T. montanum*, it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity.

The obtained values of the flavonoid concentrations in Green tea varied from 16.02 to 233.68 mg RU/g (**Fig. 2**). When flavonoid concentrations of Green tea and *T. montanum* were compared only the methanolic extracts of Green tea and methanolic of *T. montanum* showed approximately equal values. Water, acetone, ethyl acetate and petroleum ether extracts of Green tea had greater concentration of flavonoids than *T. montanum*. The flavonoid concentration in *G. biloba* standardized extract was 192.69 mg RU/g, (**Table 4**)- higher than the values of all *T. montanum* extracts.

For extraction, the solvent is chosen as a function of the type of required flavonoid. Polarity is an important consideration here. Less polar flavonoids (e.g., isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethylether, or ethylacetate, while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol-water mixtures. Glycosides have increased water solubility and aqueous alcoholic solutions are suitable (16).

DPPH scavenging activity

The antioxidant activity of different extracts of *T. montanum* was determined using methanol solution of DPPH reagent. DPPH is a very stable free radical. The effect of an antioxidant on DPPH radical scavenging is due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenylpicrylhydrazine with the loss of its violet color (18).

The antioxidant activity of twenty, whole plant and different plant part extracts of *T. montanum* is expressed in terms of IC_{50} (μg/mL) values (**Table 2**). A lower IC_{50} value indicates higher antioxidant activity. Parallel to the examination of the antioxidant activity of the plant extracts, the values for three standard compounds (**Table 3**) and two well-known medicinal plants- Green tea (**Table 2**) and *G. biloba* (**Table 4**)- were obtained and compared to the values of the antioxidant activity. The reference antioxidants used were rutin, chlorogenic acid and 3-tert-butyl-4-hydroxyanisole (BHA). Summary display for the obtained IC_{50} values of antioxidant activity of the extracts is given in **Table 2**.

The obtained values for antioxidant activity examined by DPPH radical scavenging activity ranged from 29.41 to 2408.47 μg/mL. The highest capacity to neutralize DPPH radicals was found in water extracts, especially the extract from whole plant, which neutralized 50% of free radicals at the concentration of 29.41 μg/mL. Good activity was found in methanolic, acetone and water extracts. Ethyl acetate and petroleum ether extracts showed lower capacity to inhibit DPPH radicals.

The values of antioxidant activity of Green tea obtained for comparison with *T. montanum* ranged from 14.50 to 238.25 μg/mL (**Table 2**). Comparing the antioxidant activity of Green tea and *T. montanum*, only the water extract from whole plant showed values approximate to Green tea, while the rest of

the extracts were with less activity. The value of antioxidant activity of *G. biloba* standardized extract was 33.91 µg/mL, (**Table 4**). In comparison, water extract from whole plant of *T. montanum* showed higher values than *G. biloba*, while all the other extracts of *T. montanum* showed smaller activity than *G. biloba* extracts.

TABLE 3

Values[1] of antioxidant (DPPH scavenging) activity of standard substances obtained for comparison with the values of *T. montanum*

Substances	**IC_{50} (µg/mL)**
BHA	5.39±0.31
rutin	9.28±0.27
chlorogenic acid	11.65±0.52

[1]Each value in the table was obtained by calculating the average of three analyses ± standard deviation

TABLE 4

Values[1] of phenol and flavonoid contents and antioxidant activity of *Ginkgo biloba* standardized extract obtained for comparison with the values of *T. montanum*

Type of analysis	**Values**
total phenol content (mg/g), (GAE)	140.18±0.26
concentration flavonoids (mg/g), (RUE)	192.69±0.72
antioxidant activity (IC_{50} µg/mL)	33.91±1.16

[1]Each value in the table was obtained by calculating the average of three analyses ± standard deviation

In comparison to antioxidant activity of pure standard antioxidants: BHA, rutin and chlorogenic acid (**Table 3**), water extract of *T. montanum* whole plant proved to be several times less powerful to scavenge DPPH radicals. Regarding the fact that the extract is a mixture of a great number of components opposite pure compounds used as standards, antioxidant activity of the water extract of *T. montanum* could be considered as strong.

Methanolic and acetone extracts of *T. montanum* also showed high antioxidant activity that is in accordance with their high concentration of total phenols and flavonoids.

The extraction of antioxidant substances of different chemical structure was achieved using solvents of different polarity. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentrations of phenolics in the obtained extracts using polar solvents (3). Based on results of this study, the extracts with the highest antioxidant activity had the highest concentration of phenols. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (30).

Previous investigations on *T. montanum* from Serbia led to identification of hydroxyl derivatives of benzoic (gallic, protocatechuic, gentisic, vanillic and syringic acid) and cinnammic acid (chlorogenic, caffeic, p-coumaric, ferulic and 3,5-dimethoxy-4-hydroxycinnamic acid) (31). Several stydies demonstrated the strong antioxidant activity of these phenolic acids (23, 34).

Conclusions

Based on the results of investigations, plant parts of *T. montanum* are rich sources of phenolic compounds of high value. Very effective solvents for extraction of phenolic compounds from these plants and parts of it are polar solvents. Water extract of leaves has a higher concentration of phenolic compounds compared to whole plant and to Green tea water extracts. The results indicate that water is a very efficient solvent for extraction of phenolic compounds from leaves, but for the extraction of these compounds from the whole plant methanol is suitable as solvent.

The highest concentration of flavonoids was measured in acetone and ethyl acetate leaf extracts. This information indicates effective flavonoid extraction from leaves using a moderately polar solvent.

Results suggest that the great value of *T. montanum* when it is used in pharmacy and phytotherapy, is specifically because of its usege for preparing tea. The results of this study indicate that some plant extracts have high concentrations of phenols and noticeable effect on the scavenging of free radicals equal with *Ginkgo biloba* and Green tea.

Acknowledgements

This investigation was supported by the Ministry of Science and Technological Development of the Republic of Serbia, No. III 41010. The authors are grateful to Pharmaceutical Company „Ivancic i Sinovi“, str. Palmoticeva No. 13, 11000 Belgrade, Serbia, (office@ivancic.rs), for extract of *Ginkgo biloba* support.

REFERENCES

1. **Arash R., Koshy P., Sekaran M.** (2010) J. Med. Plant. Res., **4**(3), 197-202.
2. **Bystricka J., Vollmannova A., Margitanova E., Cicova I.** (2010) Acta Agric. Slov., **95**(3), 225-229.
3. **Canadanovic-Brunet J., Cetkovic G., Dilas S., Tumbas V., Bogdanovic G., Mandic A., Markov S., Cvetkovic D. and Canadanovic V.** (2008) J. Med. Food, **11**(1), 133-143.
4. **Canadanovic-Brunet M.J., Dilas M.S., Cetkovic S.G., Tumbas T.V., Mandic I.A., Canadanovic M.V.** (2006) Int. J. Food Sci. Technol., **41**, 667-673.
5. **Chen C., Pearson M.A. and Gray I.J.** (1992) Food Chem., **43**(3), 177-183.
6. **Costa R.M., Magalhaes A.S., Pereira J.A., Andrade P.B., Valentao P., Carvalho M., Silva B.M.** (2009) Food Chem. Toxicol., **47**, 860-865.
7. **Das Sarma A., Mallick R.A., Ghosh K.A.** (2010) IJPSR, **1**(3), 185-192.

8. **Diklic N.** (1974) In: Flore de la Republique Socialiste de Serbie VI (M. Josifovic, Ed.), Acad. Serb. Sci. & Arts, Belgrade, 349-357. (in Serbian)
9. **Dilas M.S., Markov L.S., Cvetković D.D., Canadanovic-Brunet M.J., Cetkovic S.G., Tumbas T.V.** (2006) Fitoterapia, **77**, 401-403.
10. **Giovanelli G. and Buratti S.** (2009) Food Chem., **112**, 903-908.
11. **Ito N., Hirose M., Fukushima H., Tsuda T., Shairai T., Tatenastu M.** (1986) Food chem. Toxicol., **24**, 1071-1092.
12. **Kahl R. and Kappus H.** (1993) Z. Lebensm Unters Forsch, **196**(4), 329-338.
13. **Katalinic V., Milos M., Kulisic T., Jukic M.** (2006) Food Chem., **94**, 550-557.
14. **Kumarasamy Y., Byres M., Cox P.J., Jasapars M., Nahar L., Sarker S.D.** (2007) Phytother. Res., **21**, 615-621.
15. **Mariod A.A., Matthaus B., Hussein H.I.** (2007) Int. J. Food Sci. Technol., **43**(5), 921-926.
16. **Marston A. and Hostettmann K.** (2006) In: Flavonoids-Chemistry, Biochemistry and Applications (M. Andersen, K.R. Markham, Eds.), Taylor & Francis Group, London, 2-3.
17. **Min G. and Chun-Zhao L.** (2005) World Microbiol. Biotechnol., **21**, 1461-1463.
18. **Molyneux P.** (2004) Songklanakarin J. Sci. Technol., **26**(2), 211-219.
19. **Nadhala R.A., Moyo M., Staden V.J.** (2010) Molecules, **15**, 6905-6930.
20. **Panovska K.T., Kulevanova S., Stefova M.** (2005) Acta Pharm., **55**, 207-214.
21. **Pourmorad F., Hosseinimehr J.S., Shahabimajd N.** (2006) Afr. J. Biotechnol., **5**(11), 1142-1145.
22. **Quettier D.C., Gressier B., Vasseur J., Dine T., Brunet C., Luyckx M.C., Cayin J.C., Bailleul F., Trotin F.** (2000) J. Ethnopharmacol., **72**, 35-42.
23. **Rasool K.M., Sabina P.E., Ramya R.S., Preety P., Patel S., Mandal N., Mishra P.P., Samuel J.** (2010) J. Pharm. Pharmacol., **62**(5), 638-643.
24. **Shrififar F., Yassa N., Shafiee A.** (2003) Iran. J. Plant Res., **2**, 235-239.
25. **Siddique A.N., Mujeeb M., Najmi K.A., Akram M.** (2010) Afr. J. Plant Sci. **4**(1), 001-005.
26. **Singleton V.L., Orthofer R., Lamuela R.R.M.** (1999) Methods Enzymol., **299**, 152-178.
27. **Stankovic M., Topuzovic M., Markovic A., Pavlovic D., Solujic S., Niciforovic N., Mihailovic V.** (2010) Biotechnol. & Biotechnol Eq., **24**(2, special edition), 82-86.
28. **Stobiecki M. and Kachlicki P.** (2006) In: The Science of Flavonoids (E. Grotewold, Ed.), Springer Science+Business Media, New York, 47-69.
29. **Tekao T., Watanabe N., Yagi I. Sakata K.** (1994) Biosci. Biotechnol. Biochem., **58**, 1780-1783.
30. **Tosun M., Ercisli S., Sengul M., Ozer H., Polat T.** (2009) Bilogical Research, **42**(2), 175-181.
31. **Tumbas T.V., Mandic I.A., Cetkovic S.G., Dilas M.S., Canadanovic-Brunet J.** (2004) APTEFF, **35**, 265-273.
32. **Tutin T.G. and Wood D.** (1972) In: Flora Europaea III (T.G.Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb, Eds.), Cambridge Univ. Press, Cambridge, 129-135.
33. **Vukovic N., Milosevic T., Sukdolak S., Solujic S.** (2008) J. Serb. Chem. Soc., **73**(3), 299-305.
34. **Wu L.** (2007) J. Zhejiang Univ. Sci. B., **8**(9), 673-679.