Cent. Eur. J. Biol. • 5(3) • 2010 • 331–337
DOI: 10.2478/s11535-010-0022-4

VERSITA

Central European Journal of **Biology**

Antioxidant properties of *Galium verum* L. (Rubiaceae) extracts

Research Article

Neda S. Lakić[1], Neda M. Mimica-Dukić[2], Jelena M. Isak[1], Biljana N. Božin[1,*]

[1]*Department of Pharmacy, Faculty of Medicine, University of Novi Sad, 21 000 Novi Sad, Republic of Serbia*

[2]*Department of Chemistry, Faculty of Sciences, University of Novi Sad, 21 000 Novi Sad, Republic of Serbia*

Received 01 October 2009; Accepted 25 January 2010

Abstract: The antioxidant properties of methanol extracts of Lady's Bedstraw (*Galium verum* L., Rubiaceae) herb from two different localities in Serbia were evaluated. Antioxidant activity was assessed in four different model systems. Free radical scavenging capacity (RSC) was examined by measuring the scavenging activity of extracts on 2,2-diphenyl-1-pycrylhydrazil (DPPH) and hydroxyl radical (OH), as well as on hydrogen peroxide. In addition, the protective effects of lipid peroxidation (LP) in corn oil were evaluated by the TBA-assay using the Fe^{2+}/ascorbate system of induction. The amount of dried extract, the content of total phenolics, flavonoids and chlorophylls was also determined. Extracts from both locations expressed very strong scavenger activity, reducing the DPPH$^{\cdot}$ (IC_{50}=3.10 μg/ml and 8.04 μg/ml) and OH radical formation (IC_{50}=0.05 μg/ml and 0.54 μg/ml) and neutralising H_2O_2 (IC_{50}=4.98 μg/ml and 3.80 μg/ml), in a dose dependant manner. Also, examined extracts showed notable inhibition of LP (IC_{50}=11.69 μg/ml and 19.47 μg/ml). The observed differences in antioxidant activity could be partially explained by the levels of phenolics (2.44-4.65 mg and 4.57-5.16 mg gallic acid equivalents/g dry extract), flavonoids (6.38-10.70 μg and 15.56-17.96 μg quercetin equivalents/g dry extract) and chlorophylls in the investigated Lady's Bedstraw extracts.

Keywords: *Galium verum • Rubiaceae • Total phenolics • Flavonoids • Chlorophylls • Antioxidant activity • DPPH radical • OH radical • Lipid peroxidation*

1. Introduction

The phytochemicals and biochemistry of medicinal plants are of special interest for many scientists because they are widely used as nutrients, and because of the role they may play in fighting diseases of the modern age [1]. These diseases, such as malignancies, cardiovascular disorders, diabetes, rheumatism, Alzheimer`s disease and ageing processes, are often connected with oxidative stress, reactive oxygen species (ROS) and lipid peroxidation (LP) [1,2]. Antioxidants also prevent the oxidation of lipids in food and in cosmetic and pharmaceutical products as well as inhibiting undesirable microbial growth. This is of interest because both oxidation and microbial growth result in spoilage and the development of off-flavour and rancidity making the products unacceptable for human consumption. Chemicals, like sulphur dioxide, *tert*-butyl hydroxytoluene (BHT), *tert*-butyl-4-hydroxyanisole (BHA) and propyl galate (PG), can be used as antimicrobial and antioxidant agents. However, the use of some of these chemicals is restricted in several countries, as they may be dangerous to human health [3]. Since ancient times, herbs and spices derived from plants have been added to food to improve the flavour and organoleptic properties, but many of these may also act as preservatives. For this reason, recent research is concentrating on plant extracts, essential oils, fermentation products and other natural sources of antioxidants and antimicrobials. Because of this interest, there are now a number of screens for antioxidants from natural sources [4].

* *E-mail: bbozin2003@gmail.com*

Serbian flora represents an abundant resource for scientific investigation. Above all, the genus *Galium* (Rubiaceae) is represented by 37 species [5,6]. Of these, the herb Lady`s Bedstraw (*Galium verum* L.) is the most frequently used in traditional medicine. Lady's Bedstraw is a perennial herbaceous plant with golden yellow flowers that are 2-3 mm in diameter and grouped in many-flowered panicles [7]. In the past, dried plants were used to stuff mattresses because the coumarin scent of the plants acted as a flea killer. The flowers were also used to coagulate milk and, in Scotland, the plant is still used as a dye and in cheese manufacturing [8]. The herbal drug *Galii veri herba* (the upper herbaceous parts of *G. verum* ssp. *verum* collected in full blossom) is most frequently used in folk medicine as a diuretic, but it is also used as a diaphoretic, spasmolytic and for the external treatment of skin injury. Also, it is believed to have beneficial effects on nervousness, phobias, cardiovascular diseases and liver disorders [8,9]. Nevertheless, there have been no relevant investigations that substantiate these uses.

The chemical composition and type of iridoides produced by Lady`s Bedstraw have been well studied. This herb has been shown to contain speruloside, monotropein, scandoside and geniposidic acid [9-13], as well as small amounts of tannins, saponins, essential oils, waxes, pigments and vitamin C [9]. However, apart from a few very old data on the chemistry of Lady's Bedstraw phenolics [14], which are mainly responsible for some of their pharmacological effects, the antioxidant properties of Lady's Bedstraw herb remain poorly studied.

In the present study, chemical screens of methanol extracts of drug *Galii veri herba* from two locations were performed. Total phenolics, flavonoids and chlorophylls a and b were quantified and the antioxidant activity of the extracts was determined *in vitro via* the neutralization of 2,2-diphenyl-1-pycrylhydrazil (DPPH) radicals, hydroxyl (OH) radicals and hydrogen peroxide (H_2O_2), as well as by the inhibition of lipid peroxidation (LP).

2. Experimental Procedures

2.1 Plant material

In July 2007, above-ground parts of wild-growing *G. verum* ssp. *verum* in full blossom were collected at two locations in Serbia: Mt. Zlatar and Veternik (near Novi Sad). Voucher specimens of collected plants (2-1806 and 2-1807) were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad [15].

2.2 Extraction procedure

Air dried herbs were powdered (sieve 0.75) and macerated in 80% methanol (MeOH) for 24 h, 48 h and 72 h at room temperature (1:1 w/v, 100 g dried herb). After maceration, the extracts were collected, filtered and evaporated to dryness under vacuum. The quantities of dry extracts were determined gravimetrically. For the evaluation of the antioxidant activity, residues were immediately dissolved in water to make 0.1% and 1% (w/v) stock solutions.

2.3 Determination of total phenolic content

The amount of total phenolic compounds in extracts was determined spectrophotometrically in Folin-Ciocalteu (FC) reagent using the method by Fukumoto and Mazza [16] with small modifications [17]. The reaction mixture contained 500 µl 0.1% aqueous dilution of dry extract, 2.5 ml freshly prepared 0.2 M FC reagent and 2 ml 10% sodium carbonate solution. The mixture was incubated in the dark at room temperature for 30 min to complete the reaction. The absorbance of the resulting solution was measured at 760 nm on a UV-Vis spectrophotometer (Hewlett Packard 8453, Agilent Technologies, USA) using distilled water as the blank. The concentration of total phenolic compounds was expressed in mg gallic acid equivalents (GAE) per g dried extract (d.e.), using a standard curve of gallic acid (0.1-1.2 µg/ml). All measurements were replicated five times.

2.4 Estimation of total flavonoid content

Total flavonoid content in the extracts was determined spectrophotometrically according to Jia, Tang and Wu [18], using a method based on the formation of a flavonoid-aluminium complex with an absorbtivity maximum at 430 nm. Briefly, 1 ml of the aqueous dilutions of samples were mixed with 1 ml 2% $AlCl_3$ x $6H_2O$. After incubation at room temperature for 15 min, the absorbance of the reaction mixtures was measured at 430 nm. The blank sample was a 1:1 mixture of the examined extracts and distilled water. Flavonoid content was expressed in µg quercetin equivalents (QE) per g dried extract by using a standard curve of quercetin (concentration range 0.1-1.2 µg/ml). All measurements were replicated five times.

2.5 Determination of chlorophylls a and b

To quantify total chlorophylls (a + b), 80% acetone extracts were made from fresh Lady's Bedstraw and the Wettstein method was used [19]. Absorbances of acetone chlorophyll solutions were measured spectrophotometrically at 644 nm and 662 nm, against an 80% acetone blank. Total chlorophyll (a + b) content was calculated using the following equations and given

in mg/g fresh plant material:
Chla = ((9,784 x A_{662})-(0,99 x A_{644})) x V/1000 x m
Chlb = ((21,42 x A_{644})-(4,65 x A_{662})) x V/1000 x m
where A represents the measured absorbances of chlorophyll extracts at wave lenghts given in the index, V is the total volume of 80% acetone chlorophyll extracts (ml) and m is the mass of fresh plant material used for quantification. All measurements were replicated five times.

2.6 Antioxidant activity

2.6.1 Free radical scavenging capacity

Free radical scavenging capacity (RSC) of the extracts was evaluated by measuring scavenging activity on DPPH- and OH-radicals, as well as on H_2O_2 after 48 h of maceration.

2.6.1.1 DPPH assay

The DPPH assay was performed as described previously [17]. The samples (from 1 to 50 µl of the 0.1% extracts) were mixed with 1 ml 90 µM DPPH$^{\bullet}$ solution and filled to a final volume of 4 ml with 95% methanol. 1 ml 90 µM DPPH$^{\bullet}$ solution with 3 ml 95% methanol was used as a control. The absorbance of the resulting solutions, control and the blank (with the reagents only) were recorded after 1 hour at room temperature. Each sample was replicated four times. The disappearance of DPPH$^{\bullet}$ was detected spectrophotometrically at 515 nm. Percent RSC was calculated by the following equation:
RSC(%)=100% x ($A_{control}$-A_{sample})/ $A_{control}$.
From the obtained RSC(%) values the IC_{50} value, which represent the concentrations of extracts that caused 50% neutralization, was determined by linear regression analysis.

2.6.1.2 Determination of the OH radical scavenging activity

The scavenging capacity of the examined extracts for hydroxyl-radicals was evaluated by measuring the degradation of 2-deoxy-D-ribose by the OH radicals that are generated in a Fenton reaction. The degradation products are 2-thiobarbituric acid (TBA)-reactive substances, which can be determined spectrophotometrically at 532 nm [20]. Each sample was replicated four times. Blank samples were prepared in the same way but replaced the examined extracts with phosphate buffer solution. After an incubation period of 1 hour at 37°C, the extent of deoxyribose degradation was measured by the TBA reaction and the absorbance was used to calculate the inhibition rate of deoxyribose degradation by the extracts as:
RSC(%)=100% x (A_{blank}-A_{sample})/ A_{blank}
From the obtained RSC values the IC_{50} values, which represent the concentrations of extracts that caused 50% degradation, was determined by linear regression analysis.

2.6.1.3 Hydrogen peroxide scavenging activity

Scavenging activity on H_2O_2 was carried out according to the method of Ruch, Cheng and Klaunig [21]. A solution of H_2O_2 (40 mM) was freshly prepared in 0.05 M KH_2PO_4-K_2HPO_4 phosphate buffer (pH=7.4). The samples (from 2.5 to 10 µl of the 0.1% extracts) were mixed with 3.4 ml phosphate buffer and 0.6 ml 40 mM H_2O_2. The absorbance of the resulting solutions and the blank (4 ml phosphate buffer) was detected spectrophotometrically at 230 nm. The percent of H_2O_2 neutralization was calculated using the following equation:
RSC (%) = 100% × (A_{blank}-A_{sample})/A_{blank}
From the obtained RSC values the IC_{50} value, which represents the concentration of extracts that caused 50% neutralization, was determined by linear regression analysis.

2.6.2 Determination of lipid peroxidation

The extent of LP was determined by measuring the colour of adduct that was produced in reaction of TBA with malondyaldehide (MDA), as an oxidation product in the peroxidation of lipids [17].

The inhibition of lipid peroxidation was measured in an Fe^{2+}/ascorbate system of induction in corn oil. The samples (from 10-150 µl of the 1% extracts) were mixed with 30 µl corn oil, 20 µl 0.01 M $FeSO_4$ and 20 µl 0.01 M ascorbic acid, filled to 3 ml with phosphate buffer. After an hour of incubation at 37ºC, 1.5 ml TBA reagent and 0.2 ml 0.1 M EDTA were added and tubes were heated at 100°C for 15 min. After cooling the content of the MDA was determined by measuring the absorbance of the adduct at 532 nm. All reactions were replicated four times.

The percentage of LP inhibition was calculated using the following equation:
I(%) = (A_{blank}-A_{sample})/A_{blank} x 100%

2.7 Statistical analysis

The data were reported as mean values ± standard deviation (SD). Values representing the concentrations of investigated extracts that cause 50% neutralization or inhibition (IC_{50}) were determined by linear regression analysis of RSC(%) and LP inhibition results (Microsoft Excel program for Windows, v. 2000).

Samples and duration of extraction (h)		% of dried extract (g/100 g drug)	Total phenolic content (mg GAE*/g d.e.)	Total flavonoids (μg QE**/ g d.e.)	Chlorophyll (mg/g fresh extract)	
					a	b
Mt. Zlatar	24	5.69 ± 0.01	2.44 ± 0.03	6.38 ± 0.035	0.25 ± 0.01	0.46 ± 0.04
	48	7.18 ± 0.03	4.65 ± 0.03	13.40 ± 0.07		
	72	6.87 ± 0.05	3.65 ± 0.02	10.70 ± 0.05		
Veternik	24	5.67 ± 0.03	4.57 ± 0.05	17.96 ± 0.06	0.17 ± 0.01	0.31 ± 0.03
	48	7.21 ± 0.02	4.76 ± 0.02	15.56 ± 0.04		
	72	5.44 ± 0.01	5.16 ± 0.02	17.35 ± 0.07		

Table 1. Amounts of dried extracts (d.e.), total phenolics and flavonoids (± SD) of *Galii veri herba* extracts.

**GAE-gallic acid equivalents*
***Quercetine equivalents*

3. Results and Discussion

3.1 Chemical composition

Depending on the duration of extraction, between 5.44 and 7.21% (g of dried extract/100 g drug) of dried extract was obtained from plant material. The largest amount of dried extract of *Galii veri herba* was generated after the 48 h of maceration process (7.18% in Mt. Zlatar plants and 7.21% in Veternik plants). Similar but smaller amounts were obtained after the 24 h extraction (5.69 and 5.67%, respectively). However, after the 72 h extraction, notably larger amounts of dried extract were observed in the plant collected in Mt. Zlatar. The results indicate that abiotic ecological factors have no remarkable influence on the amount of dried extracts. However, the duration of extraction plays a substantial role in the amount of dry extract obtained from plant material (Table 1). Suprisingly, the longest maceration resulted in a smaller amount of dried extract, especially from the Veternik sample. This is possibly due to the fact that after 48 h all cell walls were destroyed and all plant material was present in very small particles. This could lead to adsorption of already extracted substances onto the particles, so that smaller amounts of extract would pass through filter paper, which would result in a smaller amount of dried extract after the longest period of extraction.

Total phenolics (2.44-4.65 mg and 4.57-5.16 mg GAE/g dry extract) and flavonoids (6.38-13.40 μg and 15.56-17.96 μg QE/g dry extract) were present in higher concentrations in extracts of Lady`s Bedstraw collected in Veternik (Table 1). Although one would expect to find larger amounts of phenolic substances in plants collected on a mountain, the obtained results could be related to the protective role of phenolics, especially the flavonoid aglycones, in plants collected on the outskirts of big cities such as Novi Sad. One of the functions of these biomolecules, which are produced in response to ecological stress factors like pollution, is to serve as UV-B filters in plants [22]. More investigation is required to explain the enhanced production of phenolics in certain geographic areas [23].

Total chlorophyll (a + b) content was higher in *Galii veri herba* originating from Mt. Zlatar (0.25 mg of chlorophyll a and 0.46 mg of chlorophyll b), compared to those collected in the vicinity of Veternik (0.17 mg of chlorophyll a and 0.31 mg of chlorophyll b) (Table 1). These differences can be explained by altitude and exposure to direct sunlight.

3.2 Antioxidant activity

The antioxidant activity of *Galii veri herba* extracts was evaluated in a series of *in vitro* tests. Each of these assays is based on one feature of antioxidant activity, such is the ability to scavenge free radicals or the ability to inhibit lipid peroxidation. Multiple *in vitro* methods for the evaluation of the antioxidant activities of different plant products are recommended because the composition of plant extracts is complex [24,25]. Thus, the antioxidant properties of the examined Lady`s Bedstraw extracts were evaluated both as free radical scavenging capacity (RSC) and as protective effect on the lipid peroxidation.

Source	Concentrations (μg/ml)							
	0.25	1.25	2.85	5.00	7.50	10.00	12.50	IC_{50}
Mt. Zlatar	4.66±0.07	17.58±0.15	39.01±0.26	68.27±0.29	81.54±0.23	83.82±0.15	84.18±0.20	3.10
Veternik	5.19±0.08	12.56±0.11	15.34±0.19	29.53±0.19	47.15±0.25	61.96±0.13	77.6±0.09	8.04

Table 2. Percentage of neutralization of the DPPH radical by *Galii veri herba* extracts.

Source	Concentrations (μg/ml)				
	2.50	6.25	12.50	18.75	IC_{50}
Mt. Zlatar	69.75±0.09	75.29±0.07	78.78±0.11	80.18±0.15	0.05
Veternik	65.99±0.11	73.46±0.06	81.79±0.13	85.97±0.14	0.54

Table 3. Percentage of neutralization of the OH radical by *Galii veri herba* extracts.

Source	Concentrations (μg/ml)				
	0.25	0.625	1.25	2.5	IC_{50}
Mt. Zlatar	3.06±0.25	5.19±0.15	13.83±0.13	24.89±0.04	4.98±
Veternik	31.79±0.12	34.91±0.29	38.73±0.11	43.08±0.06	3.80±

Table 4. Percentage of neutralization of H_2O_2 by *Galii veri herba* extracts.

Source	Concentrations (μg/ml)				
	2.5	6.25	12.5	18.7	IC_{50}
Mt. Zlatar	2.07±0.14	36.69±0.13	64.33±0.05	69.39±0.11	11.70
Veternik	23.91±0.12	27.11±0.08	40.55±0.15	48.25±0.07	19.47

Table 5. Inhibition of lipid peroxidation (LP) in an Fe^{2+}/ascorbate system of induction by *Galii veri herba* extracts.

In the DPPH-test, the ability of examined extracts to act as donors for hydrogen atoms or electrons to reduce DPPH$^{\cdot}$ into DPPH-H was measured spectrophotometrically (Table 2). Assessed extracts, made from *Galii veri herba* collected in Mt. Zlatar and Veternik, were able to reduce the stable radical DPPH$^{\cdot}$ to the yellow coloured DPPH-H, reaching 50% reduction with an IC_{50} of 3.10 μg/ml and 8.04 μg/ml, respectively. The DPPH values for the investigated extracts varied widely, with those from Mt. Zlatar ranging from 4.66% to 84.18% while those from Veternik ranged from 5.19% to 77.60%.

Hydroxyl radicals are created in a cascading process of reduction of molecular oxygen and are one of the most reactive and cytotoxic of all reactive oxygen species. The OH radical scavenging capacity of investigated extracts (Table 3) was measured using the deoxyribose assay. The protective effects of extracts were detected by their ability to remove from the test solution the hydroxyl radicals formed in a Fenton reaction, which prevents the degradation of 2-deoxy-2-ribose into fragments. Examined extracts inhibited the degradation of deoxyribose in dose-dependent manner, whereby OH radical neutralization values ranged from 69.75% to 80.18% for samples from Mt. Zlatar while those from Veternik ranged from 65.99% to 85.97%. Substantial inhibition of degradation was observed in all tested extract concentrations, reaching IC_{50} values at 0.05 μg/ml (Mt. Zlatar) and 0.54 μg/ml (Veternik).

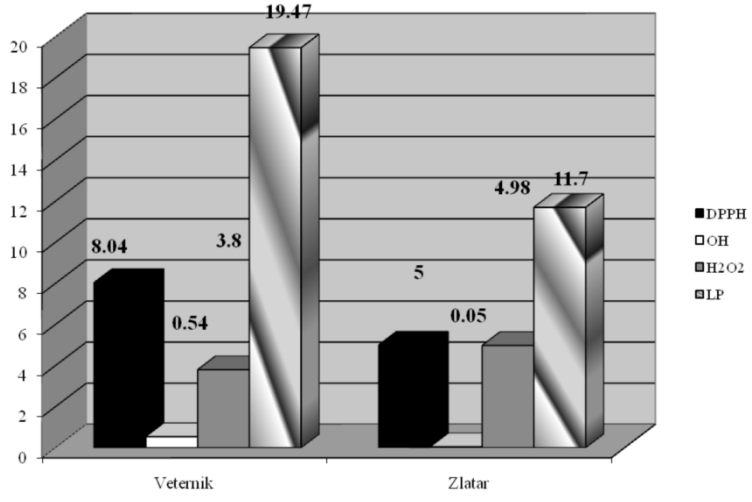

Figure 1. Antioxidant activity of examined extracts of Lady`s Bedstraw with corresponding IC_{50} values (given in μg/ml).

Although hydrogen peroxide is not a free-radical species, it is the source of the very toxic hydroxyl radical, especially in the presence of metal ions like copper or iron. Also, H_2O_2 can cross membranes and may slowly oxidise a number of cell compounds. Thus, the elimination of hydrogen peroxide, as well as the OH radical is important for both human health and the protection of pharmaceutical and food systems. The neutralization of H_2O_2 by the examined extracts was measured spectrophotometrically (Table 4). Hydrogen peroxide neutralization values varied from 3.06% to 24.89% for samples from Mt. Zlatar and from 31.79% to 43.08% for those from Veternik. The ability of the assessed extracts to neutralize H_2O_2 was dose dependent, reaching an IC_{50} of 4.98 µg/ml for *Galii veri herba* collected in Mt. Zlatar and 3.80 µg/ml for *Galii veri herba* collected in Veternik.

The stronger effects on the neutralization of DPPH (Table 2) and OH radical (Table 3) of extracts obtained from Mt. Zlatar plant material can be partially explained by the greater amount of total chlorophylls in this plant (Table 1). On the other hand, the obtained results for the H_2O_2 scavenging activity (Table 4) could be related to its greater content of phenolics and flavonoids (Table 1).

The protective effects of Lady`s Bedstraw extracts on lipid peroxidation were evaluated in an Fe^{2+}/ascorbate system of induction by the TBA-assay. The inhibition of LP was determined by measuring the formation of secondary components (malondialdehyde) of the oxidative process, using corn oil as an oxidizable substrate. However, because the thiobarbituric acid test is not specific for MDA, other non-lipid substances present in plant extracts, or peroxidation products other than malondialdehyde, could react positively with TBA [24]. These interfering compounds distort the results and therefore all the final results of investigated Lady`s Bedstraw extracts have been corrected using the absorbances of the investigated Lady's Bedstraw extracts after the TBA test (without corn oil) [26]. Inhibition values varied widely, ranging from 2.07% to 69.39% for samples from Mt. Zlatar and from 23.91% to 48.25% for those from Veternik. Both examined extracts expressed notable inhibitory activity on LP with responding IC_{50} values 11.70 µg/ml (Mt. Zlatar) and 19.47 µg/ml (Veternik) (Table 5).

4. Conclusions

The measurement of antioxidant activity was used as a method for the evaluation of Lady`s Bedstraw extracts. The comparison of antioxidant activities (IC_{50} values) of the investigated extracts (Figure 1) showed variable effects that depended on examined extracts and the model systems used for evaluation. Generally, extracts of *Galii veri herba* collected in Veternik, which proved to have higher concentrations of phenolics and flavonoids, possessed a stronger antioxidant activity only in the hydrogen peroxide neutralization assay. In contrast, extracts of Lady`s Bedstraw from Mt. Zlatar expressed stronger scavenging activity in all other tested systems, which may be related to its higher chlorophyll content.

Acknowledgements

The Ministry of Sciences and Environmental Protection, Republic of Serbia (Grant No. 142036) supported this research work.

References

[1] Van de Vijver L.P., Kardinaal A.F., Grobbe D.E., Princen H.M., Van Poppel G., Lipoprotein oxidation, antioxidants and cardiovascular risk: epidemiologic evidence, Prostaglandins, Leukotrienes and Essential Fatty Acids, 1997, 57, 479-487

[2] Smith M.A., Perry G., Pryor W.A., Causes and consequences of oxidative stress in Alzheimer's disease, Free Radic. Biol. Med., 2002, 32, 1049

[3] Safer A.M., Al-Nughamish A.J., Hepatotoxicity induced by the antioxidant food additive butylated hydroxytoluene (BHT) in rats: An electron microscopical study, Histol. Histopathol., 1999, 197, 391-406

[4] Diplock A.T., Charleux J.L., Crozier-Willi G., Kok F.J., Rice-Evans C., Roberfroid M., et al., Functional food science and defense against reactive oxidative species, Brit. J. Nutr., 1998, 80, S77-S112

[5] Gajić M., Rubiaceae B. Jusl., In: Josifović M., (Ed.), Flora of Serbia, Vol. V, Serbian Academy of Sciences and Arts, Belgrade, 1973, 463-499

[6] Nikolić V., Sigunov A., Diklić N., Appendix of the Flora of Serbia by new data for distribution plant species, In: Sarić M., (Ed.), Flora of Serbia, Vol. X, Serbian Academy of Sciences and Arts, Belgrade, 1986, 257-336

[7] Josifović M., Serbian Flora V, Serbian Academy of Arts and Science, Belgrade, 1973, (in Serbian)

[8] Bisset N.G., Wichtl M., Herbal Drugs and Phytopharmaceuticals, CRC Press, Boca Raton, London, New York, Washington D.C., 2001

[9] Tucakov J., Healing by plants, Rad, Belgrade, 2006, (in Serbian)
[10] Derek V., Banthorpe J., White J., Novel anthraquinones from undifferentiated cell cultures of Galium verum, Phytochemistry, 1995, 38, 107-111
[11] Böjthe-Horváth K., Hetényi F., Kocsis Á., Szabó L., Varga-Balázs M., Máthé I. Jr, et al., Iridoid glycosides from Galium verum, Phytochemistry, 1980, 21, 2917-2919
[12] Böjthe-Horváth K., Kocsis Á., Párkány L., Simon K., A new iridoid glycoside from Galium verum L. First X-ray analysis of a tricyclic iridoid glycoside, Tetrahedron Lett., 1982, 23, 965-966
[13] Rafaëlly L., Héron S., Nowik W., Tchapla A., Optimisation of ESI-MS detection for the HPLC of anthraquinone dyes, Dyes and Pigments, 2008, 77, 191-203
[14] Raynaud J., Mnajed H., The flavonic glycosides of Galium verum L. (Rubiaceae), Les Comptes Rendus de l'Académie des sciences, 1972, 274, 1746-1748
[15] Holmgren P.K., Holmgren N.H., Additions to Index Herbariorum (Herbaria), 8th ed., Fourteenth Series, IAPT (International Association for Plant Taxonomy), Vienna, 2003
[16] Fukumoto L., Mazza G., Asessing antioxidant and prooxidant activities of phenolic compounds, J. Agric. Food Chem., 2000, 48, 3597-3604
[17] Bozin B., Mimica-Dukic N., Samojlik I., Anackov G., Igic R., Phenolics as antioxidants in garlic (Allium sativum L., Alliaceae), Food Chem., 2008, 111, 925-929
[18] Jia Z., Tang M., Wu J., The determination of flavonoids content in mullbery and their scavenging effect on superoxide radicals, Food Chem., 1999, 64, 555-599
[19] Lisiewska Z., Kmiecik W., Slupski J., Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill (Anethum graveolens L.), depending on the time and temperature of storage, Food Chem., 2004, 84, 511-518
[20] Mimica-Dukic N., Bozin B., Sokovic M., Mihajlovic B., Matavulj M., Antimicrobial and Antioxidant Activities of Three Mentha Species Essential Oils, Planta Med., 2003, 69, 413-419
[21] Ruch R.J., Cheng S.J., Klaunig J.E., Prevention of citotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogenesis, 1989, 10, 1003-1008
[22] Kootstra, A., protection from UV-B-induced DNA damage by flavonoids, Plant Mol. Biol., 1994, 26, 771-774
[23] Harborne J.B., Phytochemical methods−a guide to modern techniques of plant analysis. Chapman & Hall, London, Weinheim, New York, Tokyo, Melbourne, Madras, 1998
[24] Chu Y.H., Chang C.L., Hsu H.F., Flavonoid content of several vegetables and their antioxidant activity, J. Sci. Food Agric., 2000, 80, 561-566
[25] Nuutila A.M., Puupponen-Pimia R., Aarni M., Oksman-Caldentey K.M., Comparision of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity, Food Chem., 2003, 81, 485-493
[26] Janero D.R., Malondiladehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury, Free Rad. Biol. Med., 1990, 9, 515-540