VERSITA

Cent. Eur. J. Biol. • 9(2) • 2014 • 182-188
DOI: 10.2478/s11535-013-0245-2

Central European Journal of **Biology**

Influence of the extraction solvent on antioxidant activity of *Althaea officinalis* L. root extracts

Research Article

Niko Benbassat[1], Krassimira Yoncheva[2]*, Vera Hadjimitova[3], Nadia Hristova[3], Spiro Konstantinov[4], Nikolai Lambov[2]

[1]*Department of Pharmacognosy, Medical University of Sofia, 1000 Sofia, Bulgaria*

[2]*Department of Pharmaceutical Technology, Medical University of Sofia, 1000 Sofia, Bulgaria*

[3]*Department of Physics and Biophysics, Medical Faculty, Medical University of Sofia, 1431 Sofia, Bulgaria*

[4]*Department of Pharmacology, Faculty of Pharmacy, Medical University of Sofia, 1000 Sofia, Bulgaria*

Received 15 February 2013; Accepted 18 July 2013

Abstract: *Althaea officinalis* (Malvaceae) is a well-known plant that is widely distributed throughout the world. Aqueous and hydroalcoholic extracts from *A. officinalis* root are used mainly because of their antitussive and expectorant activity. It is well known that these activities are based on the polysaccharide composition, but little is known about the possible antioxidant activity of root extract. The present study evaluated antioxidant activity of root extracts prepared with different extraction solvents applying $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), hypochlorous acid scavenging assay and iron-induced lipid peroxidation. The results showed that the extract prepared with water as extraction solvent did not possess antioxidant activity, whereas the extracts obtained using ethanol:water as extraction agent showed well pronounced antioxidant activity. In particular, the extracts obtained at low concentration of ethanol in the mixed solvent (50:50 and 70:30, v/v) showed higher scavenging activity for $ABTS^{\bullet+}$ radicals and hypochlorite ions than the extract obtained with the higher ethanol concentration (90:10, v/v). These results correlated very well with phenolic and flavonoid content of the extracts. The extracts did not show cytotoxic effect on human BV-173 leukemic cells but may have immunomodulating effects due to their antioxidant properties.

Keywords: *Althaea officinalis* • *$ABTS^{\bullet+}$* • *Hypochlorous acid scavenging assay* • *Lipid peroxidation* • *Total phenolic content* • *Total flavonoids*

1. Introduction

Althaea officinalis L. (genus *Althaea*) belongs to the botanical family *Malvaceae* and is widely distributed throughout the world. It is a perennial plant well-known for its healing properties since ancient time. The activity of various root extracts of *A. officinalis* against cough, throat irritation and inflammation of gastrointestinal mucosa has been described [1,2]. Syrups prepared by maceration of root (usually diluted with sucrose syrup) are used in many countries for the treatment of cough and inflammation of the mouth and pharynx. Other preparations of the plant include decoction and tea preparation [3]. Decoction is mainly administered for constipation [4], whereas infusion is used for bronchial catarrh [5]. Infusions have been reported to be applied orally for treatment of asthma and as expectorant [6,7]. Comminuted herbal substance for tea preparation is applied to alleviate abdominal aches of digestive origin. Antibacterial activity of root extract and its incorporation in mouthwash for topical periodontal prophylactics has been also reported [8]. Other studies mentioned the potential use of root extract in topical formulation due to its wound healing effects [7]. The capacity of the *A. officinalis* extracts to soothe chapped skin and to reduce the inflammation is well established. Some studies have evaluated antioxidant activity of extracts prepared from dried plant and its flowers [9,10]. Although the above-ground parts displayed a mild antioxidant activity [9,10], the antioxidant activity

* *E-mail: krassi.yoncheva@gmail.com*

of extracts from the root of *A. officinalis* remains to be determined.

The aim of the present study was to evaluate the influence of the extraction process on antioxidant activity of the extracts prepared from *A. officinalis* root. Extraction solvent, the ratio of the quantity of the herbal substance to the quantity of the resulting extract and the physical state of the herbal substance influenced the properties of the final herbal preparation [11-14]. Since the extraction solvent has a great influence on the final phytochemical composition, this factor was evaluated in the present study by using different extraction solvent.

2. Experimental Procedures

2.1 Plant material and chemicals

A. officinalis L. was cultivated and two years old roots were obtained from Botanical Garden of Sofia University "St. Kl. Ohridski" (Sofia, Bulgaria), where voucher specimen is deposited. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), pyrogallol and quercetin were obtained from *Sigma*-Aldrich. BV-173 cell line was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany).

2.2 Preparation of the extracts

The extracts were prepared using different solvents as extraction media, in particular E1 (purified water), E2 (50:50, v/v ethanol/water), E3 (70:30, v/v ethanol/water) and E4 (90:10 v/v ethanol/water). The liquid extracts were prepared at a ratio 1:10 giving a final concentration of 100 mg mL^{-1}. The extraction process included ultrasonic treatment of the soaked herbal drug into the selected medium for 2 h. The extraction was carried out at ultrasonic power of 300 W, frequency of 45 kHz and temperature of 25°C (Sonica 4300, Milano, Italy). The obtained extract was filtered trough the cellulose membrane (3.5 kDa Roth, Karlsruhe, Germany) and kept in dark glass bottles.

2.3 ABTS radical cation scavenging assay

The $ABTS^{\bullet+}$ scavenging test was used to determine the antioxidant activity. $ABTS^{\bullet+}$ radical was obtained by reaction between ABTS and potassium persulfate [15]. Blank sample was prepared from the daily solution by adding 1 mL PBS, which gives an absorbance of 0.7 ± 0.01 at 723 nm. The radical scavenging activity was assessed by mixing 2 ml of $ABTS^{\bullet+}$ solution with 1 mL PBS solutions of the investigated plant extracts with different concentrations. The reactive mixture was allowed to stand at room temperature for 10 min and the absorbance was recorded at 734 nm. The value of IC-50 was defined as the concentration of substrate that causes 50% loss of the $ABTS^{\bullet+}$ activity and was calculated as mg L^{-1}.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference compound. The Trolox equivalent antioxidant capacity (TEAC) was expressed as mol Trolox per g of sample.

2.4 Determination of total phenolic content

Phenolic content in the liquid extracts was studied using the Folin-Ciocalteu method with some modifications [16]. The extracts (0.5 mL) were mixed with 0.5 mL Folin-Ciocalteu reagent and incubated for 5 min. Then, 1 mL 20% solution of sodium carbonate was added and finally the volume was diluted to 10 ml with purified water. The mixtures were incubated for 2 h and the absorbance was measured at 765 nm (Hewllet Packard 8452A). Pyrogallol (2-15 μg mL^{-1}) was used as a standard to produce the calibration curve (r=0.9970). The mean of three readings was used and the total phenolic content was expressed as μg of pyrogallol equivalents (PE) per ml of extracts.

2.5 Determination of total flavonoid content

The total flavonoid content was determined using the method adapted from Arvouet-Grand *et al.* [17]. In the present study, 5 mL of 2% solution of $AlCl_3 \cdot 6H_2O$ in ethanol was mixed with the same volume of the extracts. The samples were incubated for 15 min and the absorbance was measured at a wavelength of 410 nm (Hewllet Packard 8452A). The total flavonoid content was calculated using a standard curve of quercetin as a standard (0–50 mg L^{-1}, r=0.9986). The total flavonoid content was expressed as μg of quercetin equivalents (QE)/ml of extracts.

2.6 Hypochlorous acid scavenging assay

The samples for the assay were prepared in 2 mL PBS (pH=7.4) and each contained 15 mmol L^{-1} taurine, 20 mmol L^{-1} potassium iodide (KI), 0.06 mmol L^{-1} sodium hypochlorite and the investigated extracts in different concentrations. Control sample did not contain extract. The samples were incubated for 30 min at room temperature and the absorbance was measured at 350 nm. The results were presented using antioxidant activity (AOA) that was calculated applying the equation: $AOA = (A_o - A)/A_o$, where A_o was the absorbance of the control and A was the absorbance of the extract samples. The values of IC-50 were defined as the concentration of the extract that caused 50% loss of the AOA and were calculated in mg mL^{-1}.

2.7 Thiobarbituric acid reactive substances (TBARS) assay

The TBARS of lipid peroxidation was measured in liposomal suspension obtained from phospholipids extracted from egg yolk. The samples were prepared in PBS and contained 1 mg lipid mL^{-1} and diluted extracts. Control sample did not contain extract. After addition of 0.1 mmol L^{-1} $FeCl_2$ the samples were incubated at 37ºC for 30 min. Then, 0.5 mL of 2.8% trichloroacetic acid and 0.5 ml of 0.5% TBA were added. The samples were heated at 100ºC for 20 min and the absorbance was measured at 532 nm. The results were presented using antioxidant property (AOP) that was calculated as $AOP=(A/A_o)x100$, where A_o was the absorbance of the control and A was the absorbance of the samples containing the extracts.

2.8 *In vitro* cytotoxicity

The cell viability after exposure to the extracts was examined applying the MTT-dye reduction assay, based on the biotransformation of the yellow dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to a violet formazan product. Exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 µL/well) at a density of 1×10^5 cells per mL and after a 24 h incubation at 37°C they were treated with graded concentrations of the extracts. The incubation after the treatment was for 72 h. After the exposure period 10 µL MTT solution (10 mg mL^{-1} in PBS) aliquots were added to each well. The microplates were further incubated for 4 h at 37°C and the MTT-formazan crystals formed were dissolved through addition of 100 µL/well 5% formic acid in 2-propanol. The MTT formazan absorption was determined using a microprocessor controlled microplate reader (Labexim LMR-1) at 580 nm.

3. Results and Discussion

Liquid formulations of *A. officinalis* (syrups, extracts, infusions) are widely used mainly due to their antitussive and anti-inflammatory activity. It is well known that these activities are based on the polysaccharide composition [18,19]. The polysaccharides possessing bioadhesive properties form a coating layer on the oral and pharyngeal mucosa that reduce the irritation associated with the dry cough [20,21]. This study evaluates the antioxidant activity of *A. officinalis* root extracts and examines the influence of the extraction solvent on this activity.

3.1 ABTS radical cation scavenging assay

The study showed that the aqueous extract of *A. officinalis* did not possess antioxidant activity. The absence of antioxidant properties of aqueous extract could be due to the extraction mainly of polysaccharides. Some polysaccharides extracted from the leaves of *A. officinalis*, in particular glucuronoxylans, showed high antioxidant activity evaluated by their ability to inhibit peroxidation of soyabean lecithin liposomes by $OH^{\bullet}$ radicals [22]. Polysaccharides extracted from the root of *A. officinalis* are mainly D-glucans, rhamnogalacturonan and arabinans [23,24]. However, a previous study reported the weak antioxidant activity of glucans [25]. Thus, the absence of antioxidant activity of the root aqueous extract in the present study corresponded with the weak activity of glucans.

The extracts obtained using ethanol:water as the extracting agent showed well pronounced antioxidant activity (AOA). The antioxidant properties of the ethanol:water extracts were investigated by concentration dependence of AOA (Figure 1). The slope of the curve is proportional to antioxidant properties of the extracts. IC-50 was calculated by using the data from Figure 1. The values of IC-50 for E2, E3 and E4 extracts were 59.6 ± 0.8 mg L^{-1}, 69.5 ± 3.9 mg L^{-1} and 313.3 ± 43.8 mg L^{-1}, respectively. The extracts obtained by low concentration ethanol solution manifested higher antioxidant activity than the extract obtained with the highest ethanol concentration (Figure 2). These results led to the suggestion that the antioxidant components were extracted more efficient at low concentration of ethanol.

Total phenolic and total flavonoid contents were determined to investigate their relation with the different antioxidant activity of the three extracts. According to literature, the root extract contains phenolic acids, in particular caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, salicylic, syringic, *p*-hydroxyphenylacetic and vanillic

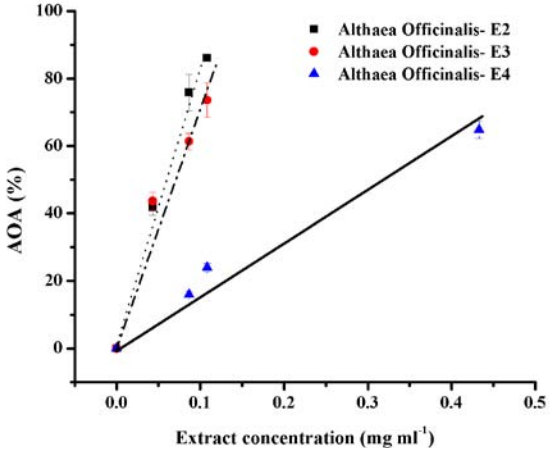

Figure 1. Relationship between concentration of the extracts (E2, E3 and E4) and their antioxidant activity (AOA). Data expressed as the mean ± SD, n=3.

acid [26]. Our results revealed that the extract received with the lowest ethanol concentration (E2) contained the maximum phenolic content. Thus, the higher phenolic content correlated with the higher antioxidant activity of the extracts (Figure 3, r=0.9903). These results could be considered in agreement with previous studies that reported antioxidant activity of phenolic acids, in particular caffeic, ferulic and p-cumaric acid [27].

Content of some flavonoids like hypolaetin-8-glucoside, isoquercitrin, kaempferol, quercetin and naringenin in the roots of *A. officinalis* has been reported [26,28,29]. The phytochemical evaluation of the extracts in the present study showed higher total flavonoid content for the extracts obtained by extraction with lower ethanol concentration in the mixed solvent (E2 and E3) (Figure 4). These results are in agreement with a study dedicated on the extraction of quercetin from onion. The authors have reported that the quercetin extracted with 60% and 90% ethanol was 298 µg L^{-1} and 207 µg L^{-1}, respectively [30]. It was considered that the reason could be the presence of quercetin in the form of glycoside having higher polarity than aglycone. In our study similar phenomenon was observed, in particular the lowest flavonoid content was found for the extract obtained with the highest ethanol concentration (E4). Further, flavonoid content in the extracts obtained by extraction with the different concentrations of ethanol correlated with the antioxidant capacity (Figure 4). As shown, the higher the flavonoid content, the higher antioxidant activity.

3.2 Hypochlorous acid scavenging assay

It is well known that activation of phagocytizing cells during inflammatory processes is associated with production of significant amounts of ROS and the neutrophils activation leads to release of hypochlorous acid in the system. For this reason, the ability of the three extracts to scavenge the hypochlorite ions was investigated. Figures 5 and 6 represent scavenging activity and the respective IC-50 values of the extracts. All extracts possessed the ability to react with hypochlorous acid. The highest effect demonstrated the extract E2 (ethanol:water - 50:50), since the value of IC-50 for this extract was the lowest – 0.84 mg mL^{-1}. IC-50 of E3 (ethanol:water - 70:30) was slightly higher than the IC-50 of E2 (1.05 mg mL^{-1}), whereas IC-50 of E4 (ethanol:water - 90:10) was significantly higher than those of E2 and E3 extracts (2.18 mg mL^{-1}). Thus, the results suggested that stronger anti-inflammatory activity would be achieved with *A. officinalis* extract prepared with ethanol:water solvent in ratio 50:50.

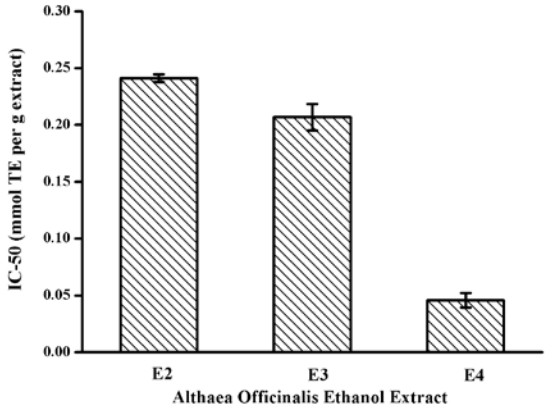

Figure 2. The antioxidant activity of the extracts (E2, E3 and E4) presented by values of IC-50 (mmol TE g^{-1} extract).

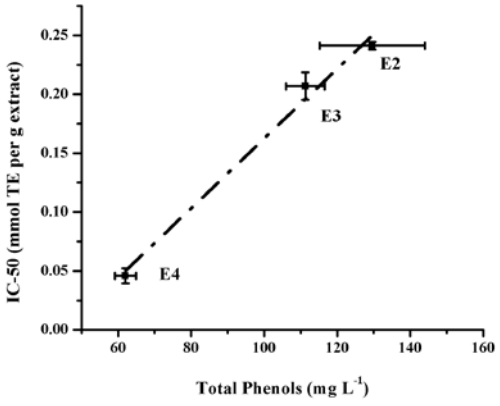

Figure 3. Relationship between total phenolic content of the extracts and antioxidant activity presented by IC-50 Trolox equivalent TE g^{-1} of the extracts. Data expressed as the mean ± SD, n=3.

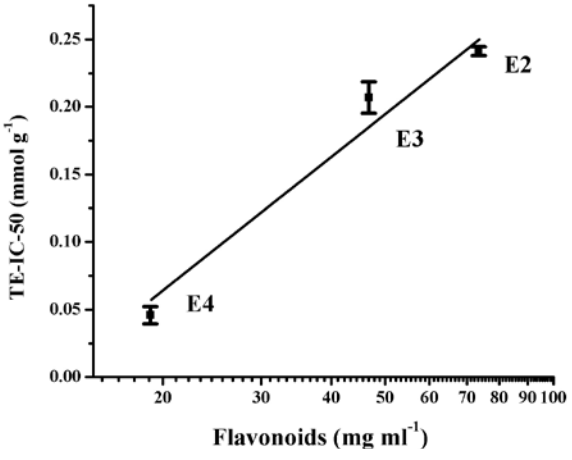

Figure 4. Relationship between total flavonoid content of extracts and antioxidant activity presented by IC-50 Trolox equivalent TE g^{-1} of the extracts. Data expressed as the mean ± SD, n=3.

3.3 Iron-induced lipid peroxidation

The antioxidant properties of the extracts in model system of lipid peroxidation *in vitro* were investigated (Figure 7). The values of IC-50 for all tested extracts were similar and got into the concentration interval 5 - 6.5 mg mL^{-1}. The observed values were 3 to 10 times higher than the values obtained by the other methods (ABTS and hypochlorite ions). Unlike the strong correlation between the radicals scavenging capacity and the flavonoid and phenolic content, the lipid peroxidation did not show such relationship. This observation could be due to the fact that in this model system the activity depended on the variety of chemical reactions including the participation of intermediate and final products.

3.4 *In vitro* cytotoxicity study

Cytotoxicity of the different extracts of *Althaea officinalis* was investigated on leukemic BV-173 cells (Figure 8). This line was selected because of its lymphoid origin and drug sensitivity despite of the expression of Bcr-Abl fusion gene which is associated with resistance toward apoptotic signaling. Previous reports found that water extract of roots (10%) was inactive on HeLa tumor cells [31]. Another study reported weak antineoplastic effect (LC_{50}>5 mg mL^{-1}) of the root extract obtained in ethanol [32]. This activity was estimated on murine neuroblastoma cells, originally derived from spontaneous malignant tumor. Water extract from *A. officinalis* root had no effect on primary dermal human fibroblasts (pNHF) but showed stimulating effect on

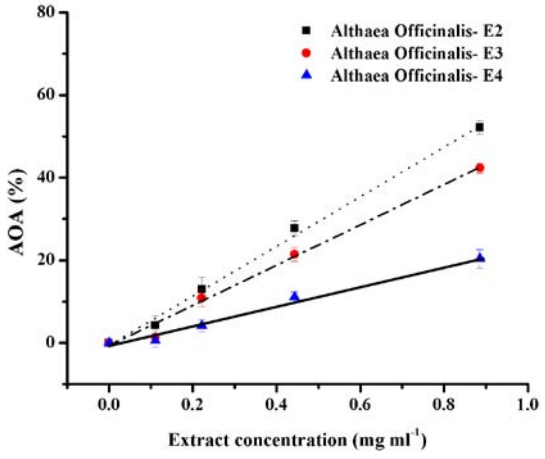

Figure 5. Relationship between concentration of the extracts (E2, E3 and E4) and their antioxidant activity detected in hypochlorite ions generating system. Data expressed as the mean ± SD, n=3.

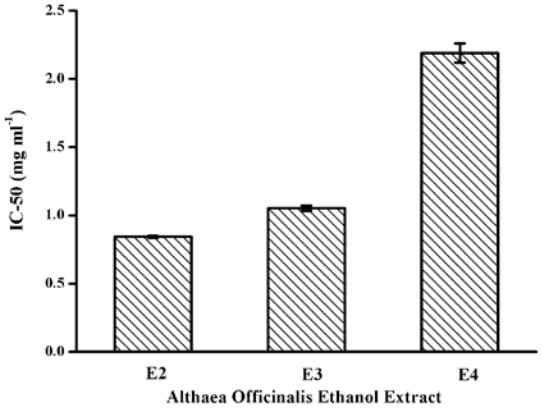

Figure 6. Antioxidant activity of the extracts in hypochlorite ions generating system presented by values of IC-50. Data expressed as the mean ± SD, n=3.

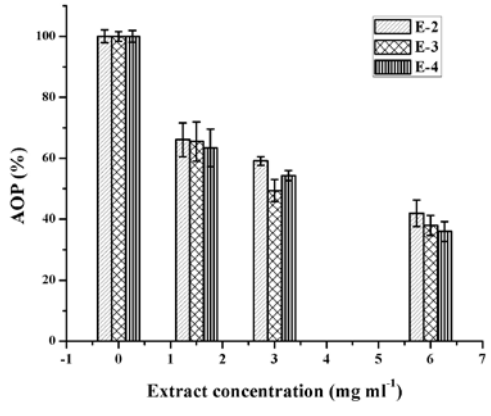

Figure 7. Antioxidant properties of *Althaea officinalis* extracts in iron-induced lipid peroxidation system. Data expressed as the mean ± SD, n=3.

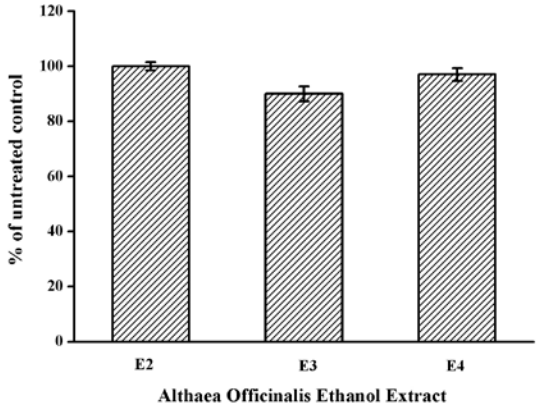

Figure 8. Cytotoxicity of the different *Althaea officinalis* extracts on leukemic BV-173 cells. Data expressed as the mean ± SD, n=3.

cell viability and proliferation of epithelial KB cells [33]. The results in the present study showed that the liquid extracts at the concentration of 50 mg mL^{-1} exerted no significant cytotoxicity on human BV-173 leukemic cells.

4. Conclusions

The present study revealed that the hydroalcoholic extract from *A. officinalis* root possesses antioxidant properties. The concentration of the ethanol in the extraction mixture influenced the antioxidant activity due to the different phytochemical composition of the extracts. The lowest ethanol concentration in the mixture led to higher content of phenolic and flavonoid content in the resulting extracts. The highest antioxidant activity correlated with the highest phenolic as well as the highest flavonoid content of the extracts. The optimal extraction regarding antioxidant activity of the final extract seemed to be with ethanol:water mixed solvent at a 1:1 ratio. Taken together our experimental findings indicate that the plant extracts do not have any antitumor properties but may have immunomodulating effects due to their antioxidant features.

References

[1] Fasse M., Zieseniss E., Bässler D., Dry irritating cough in children – a post-marketing surveillance involving marshmallow syrup, Paed., 2005, 11, 3-8

[2] Newall C., Anderson L., Phillipson J.D., Herbal Medicines. A guide for healthcare professionals, Pharmaceutical Press, London, 1996

[3] Ross I.A., Medicinal plants of the world: Chemical constituents, traditional and modern uses, Humana Press Inc., Totowa, New Jersey, Vol. 2, 2001

[4] Lokar L. C., Poldini L., Herbal remedies in the traditional medicine of the Venezia Giulia region (North East Italy), J. Ethnopharmacol., 1988, 22, 231–239

[5] De Feo V., Senatore F., Medicinal plants and phytotherapy in the Amalfitan coast, Salerno Province, Campania, Southern Italy, J. Ethnopharmacol., 1993, 39, 39–52

[6] Singh V., Traditional remedies to treat asthma in north west and Trans-Himalayan region in J. and K. States, Fitoterapia, 1995, 65, 507–509

[7] Hussey J.S., Some useful plants of early New England, Economic Botany, 1974, 28, 311–337

[8] Iauk L., Lo Bue A.M., Milazzo I., Rapisarda A., Blandino G., Antibacterial activity of medicinal plant extracts against periodontopathic bacteria, Phytother. Res., 2003, 17, 599-604

[9] Masaki H., Sakaki S., Atsumi T., Sakurai H., Active-oxygen scavenging activity of plant extracts, Biol. Pharm. Bull., 1995, 18, 162-166

[10] Elmastas M., Ozturk L., Gokce I., Erenler R., Aboul-Enein H.Y., Detremination of antioxidant activity of marshmallow flower (Althaea officinalis), Anal. Lett., 2004, 37, 1859-1869

[11] Kaurinovic B., Popovic M., Vlaisavljevic S., Trivic S., Antioxidant capacity of Ocimum basilicum L. and Origanum vulgare L. extracts, Molecules, 2011, 16, 7401-7414

[12] Leccese A., Viti R., Bartolini S. The effect of solvent extraction on antioxidant properties of apricot fruit, Cent. Eur. J. Biol., 2011, 6, 199-204

[13] Giao M.S., Pereira C.I., Pintado M E., Malcata F.X., Effect of technological processing upon the antioxidant capacity of aromatic and medicinal plant infusions: From harvest to packaging, LWT: Food Sci. Technol., 2013, 50, 320-325

[14] Zitka O., Sochor J., Rop O., Salickova S., Sobrova P., Zehnalek J., et al., Comparison of various easy-to-use procedures for extraction of phenols from apricot fruits, Molecules, 2011, 16, 2914-2936

[15] Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Rad. Biol. Med., 1999, 26, 1231-1237

[16] Singleton V.L., Orthofer R., Lamuela-Raventos R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol., 1999, 299, 152-178

[17] Arvouet-Grand A., Vennat B., Pourrat A., Legret P., Standarization and identification of the main constituents of propolis extracts [Standardisation de un extrait de propolis et identification des principaux constituants], J. Pharm. Belgique, 1994, 49, 462–468 (in French)

[18] Nosalova G., Strapkova A., Kardošova A., Capek P., Antitussive activity of an α-D-glucan isolated from the root of Althaea officinalis L., var. Robusta, Pharm. Pharmacol. Lett., 1992, 2, 195-197

[19] Nosalova G., Strapkova A., Kardošova A., Capek P., Antitussive activity of a rhamnogalacturonan isolated from the roots of Althaea officinalis L., var. Robusta, Carb. Chem., 1993, 12, 589-596

[20] Franz G., Polysaccharides in pharmacy: current applications and future concepts, Planta Medica, 1989, 55, 493-497
[21] Schmidgall J., Schnetz E., Hensel A., Evidence for bioadhesive effects of polysaccharides and polysaccharide-containing herbs in an ex vivo bioadhesion assay on buccal membranes, Planta Med., 2000, 66, 48-53
[22] Kardosova A., Machova E., Antioxidant activity of medicinal plant polysaccharides, Fitoterapia, 2006, 77, 367-373
[23] Capek P., Toman R., Kardošová A., Rosik J., Polysaccharides from the roots of the marshmallow (Althaea officinalis L.): Structure of an arabinan, Carb. Res., 1983, 117, 133-140
[24] Capek P., Toman R., Rosík J., Kardošová A., Janeček F., Polysaccharides from the roots of Althaea officinalis L.: Structural faetures of D-glucans, Collect. Czech. Chem. Commun., 1984, 49, 2674-2679
[25] Tsiapali E., Whaley S., Kalbfleisch J., Ensley H., Browder I.W., Williams D., Glucans exhibit weak antioxidant activity, but stimulate macrophage free radical activity, Free Rad. Biol. Med., 2001, 30, 393-402
[26] Gudej J., Flavonoids, phenolic acids and coumarins from the roots of Althaea officinalis, Planta Medica, 1991, 57, 284-285
[27] Kikuzaki H., Hisamoto M., Hirose K., Akiyama K., Taniguchi H., Antioxidant properties of ferulic acid and its related compounds, J. Agric. Food Chem., 2002, 50, 2161-2168
[28] Ninov S., Ionkova I., Kolev D., Constituents from roots of Althaea officinalis L. var. rusalka, Malvaceae, Fitoterapia, 1992, 43, 474-477
[29] Ionkova I., Alternative sources of biological active substances from Althaea officinalis L. var. Rusalka, CR Acad. Bulg. Sci., 1992, 9, 137-141
[30] Yoon J., Kwon Y.K., Kim H.Y., Frontiers of separation science and technology, In Z. Tong, & S.H. Kim (Eds.), Proceedings of the 4th Int. Conf. Separation Sci. Technol. (Nanning, Guangxi, China), Singapore World Scientific, 2004, 750-758
[31] May G, Willuhn G. Antiviral activity of aqueous extracts from medicinal plants in tissue cultures, Arzneim-Forsch, 1985, 28, 1-7
[32] Mazzio E., Soliman K., In vitro screening for the tumoricidal properties of international medicinal herbs, Phytother. Res., 2009, 23, 385-398
[33] Deters A., Zippel J., Hellenbrand N., Pappai D., Possemeyer C., Hensel A., Aqueous extracts and polysaccharides from Marshmallow roots (Althea officinalis L.): Cellular internalisation and stimulation of cell physiology of human epithelial cells in vitro, J. Ethnopharmacol., 2010, 127, 62-69