Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 76 (5) 709–717 (2011)
JSCS–4152
UDC *Pouzolzia zeylanica*:547.56:541.515:615.27–188
Original scientific paper

Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*

PEIYUAN LI[1]*, LINI HUO[1], WEI SU[2]**, RUMEI LU[1], CHAOCHENG DENG[1], LIANGQUAN LIU[2], YONGKUN DENG[1], NANA GUO[2], CHENGSHENG LU[1] and CHUNLING HE[1]

[1]*College of Pharmacy, Guangxi Traditional Chinese Medical University, Nanning 530001, and* [2]*College of Chemistry and Life Science, Guangxi Teachers Education University, Nanning 530001, P. R. China*

(Received 18 August, revised 1 November 2010)

Abstract: *Pouzolzia zeylanica* was extracted with different solvents (acetone, ethyl acetate and petroleum ether), using different protocols (cold-extraction and Soxhlet extraction). To evaluate the antiradical and antioxidant abilities of the extracts, four *in vitro* test systems were employed, *i.e.*, DPPH, ABTS and hydroxyl radical scavenging assays and a reducing power assay. All extracts exhibited outstanding antioxidant activities that were superior to that of butylated hydroxytoluene. The ethyl acetate extracts exhibited the most significant antioxidant activities, and cold-extraction under stirring seemed to be the more efficacious method for acquiring the predominant antioxidants. Furthermore, the antioxidant activities and total phenolic (TP) content of different extracts followed the same order, *i.e.*, there is a good correlation between antioxidant activities and TP content. The results showed that these extracts, especially the ethyl acetate extracts, could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration.

Keywords: total phenolic content; DPPH; ABTS; hydroxyl radical; reducing power; *Pouzolzia zeylanica*.

INTRODUCTION

Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases.[1–3] Although these free radicals can be scavenged by the *in vivo* produced antioxidant compounds, the endogenous antioxidants are insufficient to completely remove them and maintain a balance. As a result, dietary antioxidants are required to counteract excess free radicals.[4–7]

Corresponding authors. E-mail: *lipearpear@yahoo.cn; **aaasuwei@yahoo.com.cn
doi: 10.2298/JSC100818063L

Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are effective in their role as antioxidants, are commercially available and currently used in industrial processes. However, since suspected actions as promoters of carcinogenesis and other side effects have been reported, their use in food, cosmetic and pharmaceutical products has been decreasing.[8–12]. Thus, there has been an upsurge of interest in naturally-occurring antioxidants from vegetables, fruits, leaves, oilseeds, cereal crops, tree barks, roots, spices and herbs [13–16].

Pouzolzia zeylanica (L.) Benn. is a perennial herbaceous plant belonging to the Urticaceae family.[17] It is used as a remedy for diarrhea, indigestion, infantile malnutrition, urination difficulties and injuries from falls. Moreover, it is especially useful in conditions such as acute mastitis and pyogenic infections.[2,3] However, no chemical and biochemical information concerning *P. zeylanica* has been reported.

In the present study, the antiradical and antioxidant activities of the whole plant of *P. zeylanica* in four *in vitro* models, including DPPH, ABTS and hydroxyl radical scavenging assays and the reducing power assay, were investigated The total phenolic (TP) content and the relationships between the TP content and antioxidant activities were also investigated.

EXPERIMENTAL

Sample and reagents

Pouzolzia zeylanica whole plant was collected during the summer of 2009 in the Guangxi province, China. A voucher specimen was identified by Dr. Songji Wei at the Department of Zhuang Pharmacy, Guangxi Traditional Chinese Medical University. 2,2'-Azinobis(3--ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich, St. Louis, USA. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (purity 98 %) was purchased from Wako Chemicals, Japan. Gallic acid standard was purchased from J & K Scientific Ltd., Beijing, China. Other chemicals were obtained from the China National Medicine Group Shanghai Corporation, Shanghai, China. All employed chemicals and solvents were of analytical grade.

Preparation of extracts

The extraction of *Pouzolzia zeylanica* was performed using two different methods: *i*) cold-extraction under magnetic stirring and *ii*) Soxhlet extraction. For each extraction method, three different solvents were used: acetone, ethyl acetate and petroleum ether (boiling point range 60–90 °C).

Cold-extraction under stirring. Fifty grams of air-dried plant material was extracted with 500 mL of the individual solvents under constant stirring. The filtrate was collected three times at 48 h intervals during a total extraction period of 144 h. The acetone extract (CAE), ethyl acetate extract (CEE) and petroleum ether extract (CPE) were obtained by concentrating the extract liquid under reduced pressure at 40 °C using a vacuum rotary evaporator and the dry extracts were stored at –20 °C until use.

Soxhlet extraction. Ten grams of *P. zeylanica* material was extracted with 100 mL of the individual solvents using a Soxhlet apparatus for 7 h. The extract liquid was then concentrated

in a vacuum rotary evaporator at 40 °C and the obtained dry extracts (SAE, SEE and SPE) were stored at –20 °C until use.

Determination of total phenolic content

Total phenolic (*TP*) concentration in the extracts was determined using Folin–Ciocalteu reagent (FCR), according to the method of Kumar *et al.*[18] with slight modification. Gallic acid was used as a standard. Briefly, the solution of each extract (0.5 mL, 1 mg mL^{-1}) was diluted to 10 mL with distilled water in a volumetric flask. FCR (1 mL) was added and mixed thoroughly, and then sodium carbonate solution (3 mL, 2 %) was added. The absorbance at 760 nm was measured after 2 h. The total phenolic content was determined by comparison with the standard calibration curve of gallic acid, and results are presented as micrograms of gallic acid equivalents (mg of GAE) per gram dry weight (g DW). All tests were conducted in triplicate.

DPPH radical scavenging assay

Plant extracts were tested for the scavenging effect on DPPH radical according to the method of Pan *et al.*[19] 0.2 mL of extract solution in ethanol (95 %) at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}) was added to 8 mL of 0.004 % (w/v) stock solution of DPPH in ethanol (95 %). The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a UV–Visible TV--1901 spectrophotometer (Beijing Purkinje General Instrument Co. Ltd., China). As a positive control, synthetic antioxidant BHT was used. All determinations were performed in triplicate. The DPPH radical scavenging activity (*S%*) was calculated using the following equation: $S\% = ((A_{control} - A_{sample})/A_{control})\times100$, where $A_{control}$ is the absorbance of the blank control (containing all reagents except the extract solution) and A_{sample} is the absorbance of the test sample.

ABTS radical scavenging assay

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical scavenging activity following the procedure described by Delgado-Andrade *et al.*[20] Briefly, $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 730 nm of 0.70±0.02. After the addition of 10 μL of sample to 4 mL of diluted $ABTS^{\bullet+}$ solution, the absorbance was measured at 30 min. All samples were analyzed in triplicate. The $ABTS^{\bullet+}$ radical-scavenging activity of the samples was expressed as $S\% = ((A_{control} - A_{sample})/A_{control}))\times100$, where $A_{control}$ is the absorbance of the blank control ($ABTS^{\bullet+}$ solution without test sample) and A_{sample} is the absorbance of the test sample.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method of Beara *et al.*[21] with some modification. 2 mL of extract solution (0.2, 0.5, 0.8 and 1.2 mg mL^{-1}), 1.0 mL of *ortho*-phenanthroline (7.5 mmol L^{-1}), 5.0 mL of phosphate buffer (0.2 M, pH 6.6), 1.0 mL of ferrous sulfate (7.5 mmol L^{-1}) and 1.0 mL of H_2O_2 (0.1 %) were mixed and diluted to 25 mL with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm. The scavenging percentage (*P%*) was calculated as $P\% = ((A - A_1)/(A_2 - A_1))\times100$, where A, A_1 and A_2 are the absorbance value of the system with all solution including H_2O_2 and the extract solution, the system without extract solution, and the system without H_2O_2 and the extract solution, respectively.

Measurement of the reducing power

The reducing power was determined as described by Gulcin.[7] Briefly, 120 μL of extract solution at different concentrations was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 7.4) and 2.5 mL of potassium ferricyanide (1 %). After the mixture had been incubated at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10 %, w/v) was added, and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5 mL aliquot of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1 %), and then the absorbance was measured at 700 nm. The higher the absorbance value the stronger is the reducing power. All measurements were made in triplicate.

Statistical analysis

All tests were conducted in triplicate. The results are expressed as means±*SD*. Analysis of variance and significant differences among the means were tested by the one-way ANOVA, using SPSS (Version 13.0 for Windows, SPSS Inc., Chicago, IL). Values of $P < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Total phenolic (TP) content

It is well known that phenolic compounds are potential antioxidants and free radical-scavengers; hence, there should be a close correlation between the content of phenolic compounds and antioxidant activity.[18] In the present study, the TP content of various solvent extracts from *Pouzolzia zeylanica* was investigated. The results are given in Table I ($P<0.01$). The TP content varied in the different extracts and ranged from 38.9 to 90.5 mg GAE g^{-1} DW. The extract with the highest TP content was CEE (90.5 mg GAE g^{-1} DW), followed by SEE (81.2 mg GAE g^{-1} DW), indicating that CEE might have the most outstanding antioxidant activity. The TP contents were in the following order: CEE > SEE > CAE > SAE > CPE > SPE.

TABLE I. TP content of various extracts from *Pouzolzia zeylanica*. Results are the mean±*SD* of three parallel measurements. The values bearing different letters are very significantly different ($P < 0.01$)

Sample	Total phenolics, mg GAE g^{-1} DW
CAE	73.6 ± 0.16^{c}
SAE	69.2 ± 0.25^{d}
CEE	90.5 ± 0.28^{a}
SEE	81.2 ± 0.32^{b}
CPE	39.4 ± 0.20^{e}
SPE	38.9 ± 0.13^{e}

DPPH radical scavenging activity

DPPH has been widely used for free radical-scavenging assessments due to its ease and convenience. In the present study, all extracts were found to be effective scavengers against DPPH radical. They were superior to BHT and their activities increased in a concentration dependent manner (Fig. 1). The ethyl ace-

tate extracts showed the highest DPPH radical scavenging activity, while the weakest scavengers were the petroleum ether extracts. On the other hand, the extracts obtained by cold-extraction exhibited stronger DPPH radical scavenging ability than the corresponding extracts obtained by Soxhlet extraction. For instance, CEE possessed a scavenging capacity of 64.9 % on the DPPH radical, whereas that of SEE was only 55.9 %.

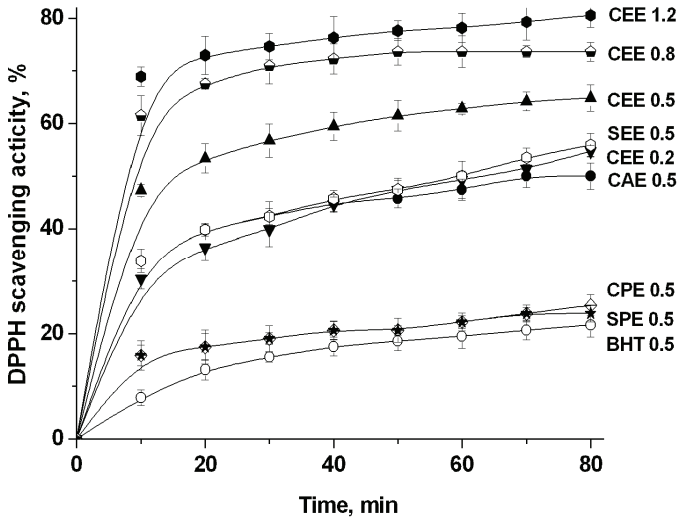

Fig. 1. DPPH radical scavenging activity of the various solvent extracts from *Pouzolzia zeylanica* compared with BHT. The values are the mean ± *SD* of three parallel measurements. The values are significantly different ($P < 0.05$) when compared to the control.

ABTS radical scavenging activity

The scavenging capacities of the various extracts for the ABTS radical were measured and compared (Fig. 2). As can be seen, the scavenging effect of all extracts increased with increasing concentration. As in the case of DPPH radical scavenging, CEE exhibited the highest ABTS antiradical properties, followed by CAE with an inhibition of 50.3 % for the ABTS radical at 1.2 mg mL^{-1}. In addition, SEE possessed a strong scavenging capacity for the ABTS radical, which was a little lower than that of CEE. The order of ABTS radical scavenging activity of all extracts was similar to that observed for DPPH. The differences in the ABTS scavenging activities exhibited by the various extracts indicated that the extracting solvent and extraction method influenced the antioxidant ability of the extracts.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the various extracts was investigated (Fig. 3). All extracts exhibited strong concentration-dependent scavenging

abilities for the hydroxyl radical. CEE was found to be the most powerful scavenger of the hydroxyl radical, with an inhibition of up to 90.5 % at a concentration of 1.2 mg mL^{-1}. It is worth mentioning that CEE showed an inhibition of 10.9 % at a concentration as low as 0.2 mg mL^{-1}. The weakest scavenger was found to be SPE, the inhibition of which, however, reached 52.3 % at 1.2 mg mL^{-1}. The results showed that the extracts obtained by both cold-extraction and by Soxhlet extraction had excellent scavenging activities for the hydroxyl radical. Furthermore, the order of antiradical ability for the hydroxyl radical was similar to those for ABTS and DPPH radicals and the TP content.

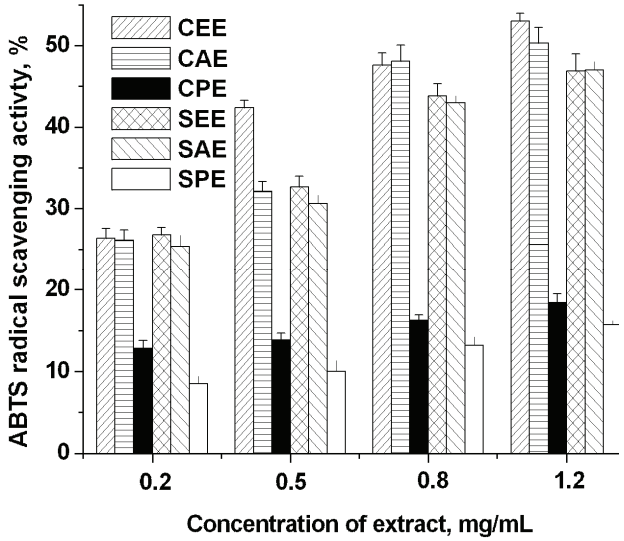

Fig. 2. ABTS radical scavenging activity of the various solvent extracts from *Pouzolzia zeylanica*. The values are the mean±*SD* of three parallel measurements. The values are significantly different ($P < 0.05$) when compared to the control.

Reducing power

The reducing powers of the various solvent extracts from *Pouzolzia zeylanica* are shown in Fig. 4. Different extracts exhibited different degrees of electron donating capacities in a concentration-dependent manner, whereby CEE was the most outstanding at the various concentrations. The reducing capacities at 700 nm for CEE, CAE, CPE and SEE were 1.14, 0.72 0.38 and 0.93, respectively. Therefore, reducing power order was: CEE > SEE > CAE > CPE. The trend in reducing power of the various solvent extracts from *Pouzolzia zeylanica* was similar to that observed for DPPH, ABTS, hydroxyl radical scavenging activities and the content of TP, indicating that there is a correlation between the TP content and the antioxidant activities of the various solvent extracts from *Pouzolzia zeylanica.*

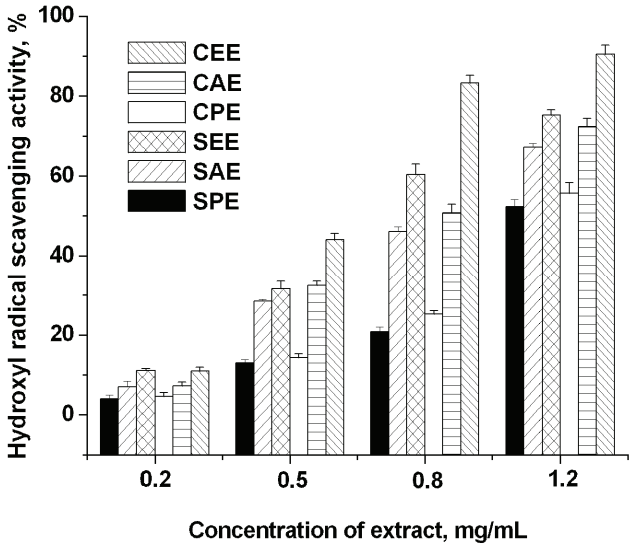

Fig. 3. Hydroxyl radical scavenging activity of the various solvent extracts from *Pouzolzia zeylanica*. The values are the mean±*SD* of three parallel measurements. The values are significantly different ($P < 0.05$) when compared to the control.

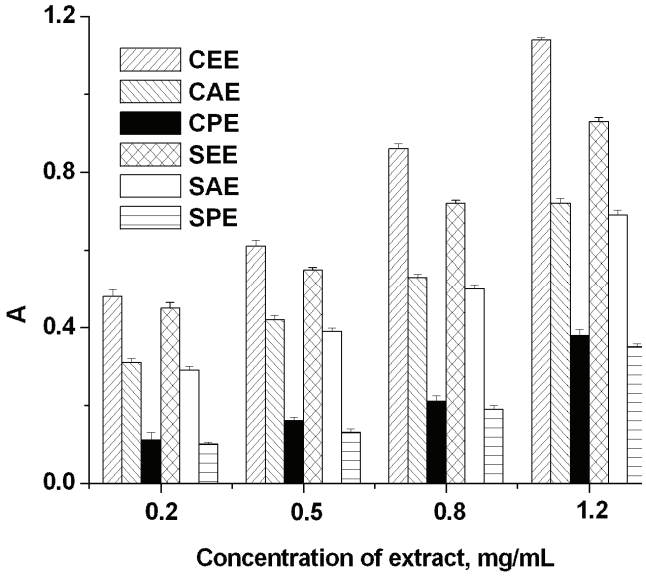

Fig. 4. Reducing power of the various solvent extracts from *Pouzolzia zeylanica*. The values are the mean±*SD* of three parallel measurements. The values are significantly different ($P < 0.05$) when compared to the control.

CONCLUSIONS

In the present investigation, extracts of *Pouzolzia zeylanica* exhibited outstanding scavenging effects on DPPH, ABTS and hydroxyl radicals, and pronounced reducing powers. It is one of the few members of the Urticaceae family which have been investigated for their antioxidant activities and showed a high antioxidant capacity compared to the intensive research of members of other families, such as Lamiaceae, Asteraceae, Fabaceae, Geraniaceae and Rosaceae. CEE proved to be the most efficient extract and was superior to butylated hydroxytoluene. It contained the highest total phenolic content (TP) of 90.5 mg g^{-1} DW, expressed as the gallic acid standard (determined by the Folin-Ciocalteu method). The TP content and antioxidant activities in both tested systems of different extracts followed the same order: CEE > SEE > CAE > SAE > CPE > SPE, showing there were significant correlations between the antioxidant activities and the TP content of *Pouzolzia zeylanica*. The results indicated that all extracts from *P. zeylanica*, obtained by cold-extraction under stirring or Soxhlet extraction, contained phenolic compounds and exhibited excellent antioxidant activities. However, cold-extraction under stirring seemed to be the more efficacious method of acquiring antioxidants exhibiting capacities. Since this investigation is a preliminary study, a detailed study of the antioxidant mechanisms of specific phenolic components is an absolute necessity, and is in progress. Nevertheless, based on the above presented results, various solvent extracts of *P. zeylanica*, especially the CEE extract, could be investigated as a possible new source of natural antioxidants in the food, nutraceuticals and cosmetic industry.

Acknowledgements. The authors thank the National Natural Science Foundation of China (Grant No. 20961001), the Key Project of the Chinese Ministry of Education. (Grant No. 2010168), the Guangxi Natural Science Foundation (Grant No. 2010GXNSFB013014), the Scientific Research Fund of Guangxi Provincial Education Department (Grant No. 200911MS146) for financial support.

ИЗВОД

КАПАЦИТЕТ УКЛАЊАЊА СЛОБОДНИХ РАДИКАЛА, АНТИОКСИДАТИВНА АКТИВНОСТ И САДРЖАЈ ФЕНОЛА У *Pouzolzia zeylanica*

PEIYUAN LI[1], LINI HUO[1], WEI SU[2], RUMEI LU[1], CHAOCHENG DENG[1], LIANGQUAN LIU[2], YONGKUN DENG[1], NANA GUO[2], CHENGSHENG LU[1] и CHUNLING HE[1]

[1]*College of Pharmacy, Guangxi Traditional Chinese Medical University и* [2]*College of Chemistry and Life Science, Guangxi Teachers Education University, Nanning, China*

Састојци *Pouzolzia zeylanica* су екстраховани различитим растварачима (ацетон, етил-ацетат, петролетар) применом две методе (хладна екстракција и Сокслетовом апаратуром). За процену антирадикалске и антиоксидативне способности екстраката коришћена су четири *in vitro* система: DPPH, ABTS, тест уклањања хидроксилних радикала и тест одређивања редукционе способности. Сви екстракти су испољили изузетну антиоксидативну активност, која је била већа и од активности бутилованог хидрокси-толуена. Екстракти у етил-ацетату

су имали највећу антиоксидативну активност, а метода хладне екстракције уз мешање је била најефикаснија за изоловање антиоксиданаса. Антиоксидативна активност је била у директној корелацији са укупним фенолним садржајем екстраката. Резултати су показали да ови екстракти, а нарочито етил-ацетатни, могу послужити као извор природних антиоксиданаса који се могу користити за терапију обољења насталих као последица оксидативних реакција у организму.

(Примљено 18. августа, ревидирано 1. новембра 2010)

REFERENCES

1. M. M. Al-Dabbas, T. Suganuma, K. Kitahara, D. X Hou, M. Fujii, *J Ethnopharmacol.* **108** (2006) 287
2. R. Tsao, Z. Deng, *J. Chromatogr. B* **812** (2004) 85
3. E. Hayet, M. Maha, A. Samia, M. Mata, P. Gros, H. Raida, M. M. Ali, A. S. Mohamed, L. Gutmann, Z. Mighri, A. Mahjoub, *World J. Microbiol. Biotechnol.* **24** (2008) 2933
4. Y. Y. Lim, J. Murtijaya, *LWT-Food Sci. Technol.* **40** (2007) 1664
5. A. Scalbert, C. Manach, C. Morand, C. Remesy, *Crit. Rev. Food Sci. Nutr.* **45** (2005) 287
6. K. Wang, Y. Pan, H. Wang, Y. Zhang, Q. Lei, Z. Zhu, H. Li, M. Liang, *Med. Chem. Res.* **19** (2010) 166
7. I. Gulcin, R. Elias, A. Gepdiremen, K. Taoubi, E. Koksal, *Wood Sci. Technol.* **43** (2009) 195
8. M. Namiki, *Crit. Rev. Food Sci.* **29** (1990) 273
9. O. Politeo, M. Jukic, M. Milos, *Food Chem.* **101** (2007) 379
10. B. Tepe, D. Daferera, A. Sokmen, M. Sokmen, M. Polissiou, *Food Chem.* **90** (2005) 333
11. C. S. Ku, S. P. Mun. *Bioresour. Technol.* **99** (2007) 2852
12. I. Gulcin, M. E. Buyukokuroglu, M. Oktay, O. I. Kufrevioglu, *J. Ethnopharmacol.* **86** (2003) 51
13. T. M. Rababah, N. S. Hettiarachy, R. Horax, *J. Agric. Food Chem.* **52** (2004) 5183
14. N. Gámez-Meza, J. A. Noriega-Rodríguez, L. Leyva-Carrillo, J. Ortega-García, L. Bringas-Alvarado, H. S. García, L. A. Medina-Juárez, *J. Food Process. Pres.* **33** (2009) 110
15. G. Jiang, Y. Jiang, B. Yang, C. Yu, R. Tsao, H. Zhang, F. Chen, *J. Agric. Food. Chem.* **57** (2009) 9293
16. M. Terashima, I. Nakatani, A. Harima, S. Nakamura, M. Shiiba, *J. Agric. Food. Chem.* **55** (2007) 165
17. Z. Wu, D. Hong, *Flora of China*, Vol. 5, Science Press, Beijing, China, 2003, p. 175
18. K. S. Kumar, K. Ganesan, P. V. Subba Rao, *Food Chem.* **107** (2008) 289
19. Y. Pan, K. Wang, S. Huang, H. Wang, X. Mu, C. He, X. Ji, J. Zhang, F. Huang, *Food Chem.* **106** (2008) 1264
20. C. Delgado-Andrade, J. A. Rufián-Henares, F. J. Morales, *J. Agric. Food Chem.* **53** (2005) 7832
21. I. N. Beara, M. M. Lesjak, E. D. Jovin, K. J. Balog, G. T. Anackov, D. Z. Orcić, N. M. Mimica-Dukić. *J. Agric. Food Chem.* **57** (2009) 9268.