Asian Journal of Chemistry; Vol. 23, No. 12 (2011), 5280-5284

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Study on the Phenolic Content, Antioxidant and Antimicrobial Effects of *Sternbergia clusiana*

R. MAMMADOV, Y. KARA* and H. ERTEM VAIZOGULLAR

Department of Biology, Faculty of Science and Arts, Pamukkale University, 20100 Denizli, Turkey

*Corresponding author: Fax: +90 258 2963535; Tel: +90 258 2963669; E-mail: eylul@pau.edu.tr

(*Received*: 27 October 2010; *Accepted*: 12 August 2011) AJC-10269

In this study, the antioxidant, antimicrobial effects and phenolic content of *Sternbergia clusiana* were investigated. Antimicrobial activity was determined by agar-well diffusion method. The total phenolic content of extracts was determined using to the Folin-Ciocalteu method. Total antioxidant activity was evaluated by β-carotene-linoleic acid method. The highest phenolic content was found in bulb-methanol extract. Results showed that the highest antioxidant activity was in the bulb-ethanol solution and the least antioxidant activity was in the bulb-benzene solution. According to free radical scavenging, the values of bulb-methanol, bulb-benzene and leaf-acetone were found to be higher than the values of butylated hydroxytoluene (BHT). Sternbergia bulb ethanol (SBE), sternbergia bulb acetone (SBA), sternbergia leaf ethanol (SLE) and sternbergia bulb benzene (SBB) extracts have showed mostly effect on G(+) bacteria. Sternbergia bulb ethanol and sternbergia bulb acetone extracts have showed mostly effect on G(–) bacteria. Extracts of *S. clusiana* are quite effective on *Candida albicans* ATCC 10239 except for sternbergia leaf methanol (SLM). In this study, extracts of *S. clusiana* have showed antioxidant and antimicrobial activity.

Key Words: *Sternbergia clusiana*, Phenolic content, Antioxidant, Antimicrobial, Extract, Radical-scavenging.

INTRODUCTION

Antioxidants are the compounds that, when added to food products, especially to lipids and lipid-containing foods, can increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants since the beginning of 20th century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity[1].

The application of bioactive plant components may increase the stability of foods and at the same time, improve their healthy properties associated with anticancer, anti-allergic and antiinflamatory activities of poly phenols in the human body[2,3].

Therefore, a natural antioxidant derived from plants has gained importance and the scientific community in searching for new therapeutic alternatives has studied many kinds of plants[3-6]. Recently, there has been considerable interest in extracts from plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods[7,8]. The species of Sternbergia always take the attention of scientists; therefore different studies are done on this species[9-15]. The genus of Sternbergia belongs to the family Amaryllidaceae. Sternbergia is represented by 6 taxa in Turkey. Plants of the family Amaryllidace are well known not only for their ornamental value but also for the alkaloids they produce. Some of these alkaloids exhibit interesting pharmacological and/or biological properties[14]. In this study, we evaluated the antioxidant and antimicrobial effects and total phenolic compounds of *Sternbergia clusiana*.

EXPERIMENTAL

Different sections (leaves and bulbes) of *Sternbergia clusiana* Ker-Gawl ex Sprengel were collected from the natural environment of the province of Mugla (C2 Mugla: Bayir and Kavaklidere village, 600-700 m) in Turkey[16].

Methanol, ethanol, benzene, acetone, β-carotene, chloroform, linoleic acid, Tween 20, distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and all other chemicals and solvents were analytical grade.

Plant extracts: The moment that the plants, which had blossoms, were collected, its bulbs and leaves were dried, chopped up with a blender and prepared for the experiment. In this study, 10 g of the plant and 100 mL of solvent (Merck) were used for each sample. These extractions were prepared using different solvents (70 % methanols, 70 % ethanol, acetone and benzene). The mixture was extracted after being heated in a vibrating water bath at 55 ºC. Having been acquired as a result of extraction, the mixture filtered through filter

paper (Whatman No: 1) and the solvents were evaporated in a rotary evaporator at 48-49 °C. The water in each extract was frozen in freeze-drying machine and then drawn out. After that, the capacity of extracts is calculated (Table-1). Extracts obtained from *Sternbergia clusiana* as follows: Sternbergia (S), bulb-methanol (SBM), bulb-ethanol (SBE), bulb-acetone (SBA), bulb-benzene (SBB), leaf-methanol (SLM), leaf-ethanol (SLE), leaf-acetone (SLA) and leaf-benzene (SLB) extracts were used in the study. Plant extracts stored in -20 ºC until further use.

TABLE-1
EFFICIENCY OF *S. clusiana* PLANT EXTRACTS (%)

Extracts	Methanol	Ethanol	Acetone	Benzene
SB*	6.73	4.11	3.83	1.15
SL**	2.84	1.47	3.73	1.13

*SB: *S. clusiana* bulbs, **SL: *S. clusiana* leaves.

Phenolic content: The total phenolic content (µg of PEs[a]/mg of extract) of extracts was determined using to the Folin-Ciocalteu method[17,18]. Briefly, 0.75 mL of Folin-Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 100 mL of sample (5 mg/mL) were put into a test tube. The mixture was mixed and allowed to stand for 5 min at room temperature. The mixture was allowed to stand at room temperature for 5 min. 0.75 mL of 6 % (w/v) Na_2CO_3 was added to the mixture and then mixed gently. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 725 nm. The standard calibration (0.01-0.05 mg/mL) curve was plotted using ferulic acid. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per 100 g plant extract.

Antioxidant activity

β-Carotene-linoleic acid assay: Antioxidant activity of plant extracts were measured according to the method described by Amin and Tan[19]. One mL of β-carotene solution (0.2 mg/mL chloroform) was pipetted into a round-bottom flask (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of 100 % Tween 20. The mixture was then evaporated at 40 ºC for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 mL of distilled water. The distilled water was added slowly to the mixture and agitated vigorously to form an emulsion. 4.8 mL of this emulsion was placed into test tubes which had 0.2 mg of the sample and 0.2 of the extract in them. For control, 0.2 mL of solvent (70 % methanols, 70 % ethanol, acetone or benzene) was placed in test tubes instead of the extract. As soon as the emulsion was added into the test tubes, initial absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Japanese) to be at 470 nm. The measurement was carried out at 0.5 h intervals for 2 h. All samples were assayed in triplicate. The antioxidant activity was measured in terms of successful bleaching of β-carotene by using the following equation.

The measurements were made using the equation below:

$$\text{Antioxidant activity} = \left(1 - \frac{A_0 - A_t}{A°_0 - A°_t}\right) \times 100$$

where A_0 and $A_0°$ are the absorbance values measured at the initial incubation time for samples and control, respectively, While A_t and $A_t°$ are the absorbance values measured in the samples or standards and control at $t^{½}$ 2 h.

Determination of free radical scavenging activity: Effect of extract on DPPH free radical was measured based on Lee *et al*.[20] method. Free radical scavenging activity of the extracts was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)[21,22]. This spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the extracts in ethanol was added to 4 mL of 0.004 % methanol solution of DPPH. After a 0. 5h incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in per cent (I %) was calculated in following way:

$$I(\%) = \left(\frac{A_{blank} - A_{Sample}}{A_{blank}}\right) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration. Tests were carried out in triplicate.

Microorganisms: The activities of methanol, ethanol, acetone and benzene extract of *S. clusiana* were measured against the following cultures: *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (ATCC 12598), *Pseudomonas aeruginosa* (NRRL B-23), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (RSKK 863), *Candida albicans* ATCC 10239. ATCC were obtained from The American Type Culture Collection. RSKK were obtained from the Culture Collection of Refik Saydam Centra Hygine Institue.

Determination methods of antimicrobial activity: Antimicrobial activity of the methanol, ethanol, acetone and benzene extract was *S. clusiana* determined by the agar-well diffusion method. The diffusion method was implemented[23,24]. The bacteria strains were cultured into Nutrient Broth and incubated at 37 ºC for 24 h and the yeast was cultured into Sabouraud Dextrose Broth and incubated at 28 ºC for 48 h. The extracts, which were obtained from the bulbs and leaves of the *S. clusiana* plant using methanol, ethanol, acetone and benzene, was applied on bacteria strains to be a concentration at 1 mg/mL that have been prepared for experiment. For the control, discs that only had solvents were prepared. These discs were placed on petri dishes. The plates were incubated at 37 ºC for 24 h and the yeast at 28 ºC for 48 h.

RESULTS AND DISCUSSION

The total phenolic content of the plant extracts is between 250.1-1126.9 mg GAE/100 g shown in Table-2. *S. clusiana* extract absorbance were measured at 470 nm with 0.5 h intervals (0, 30, 60, 90 and 120).

The extracts and control efficiencies were shown in Figs. 1 and 2. Absorbance efficiency of SLM, SBM, SBE, SLE, SBA, SLA, SLB and SBB extracts were higher than absorbance efficiency of controls.

TABLE-2
TOTAL PHENOLIC CONTENT OF EXTRACTS FROM DIFFERENT PARTS OF *S. clusiana* USING VARIOUS SOLVENTS

Variety	Total phenolic content*
SBM	1126.0 ± 18,3
SBE	1027.3 ± 18.5
SBA	716.4 ± 12.9
SLA	510.7 ± 16.2
SLM	412.3 ± 21.4
SLE	362.7 ± 10.3
SLB	281.3 ± 11.2
SBB	250.1 ± 7.1

*mg gallic acid equivalent (GAE)/100 g sample, Each value is expressed as the mean ± standard deviation (n = 6). ($p < 0.05$).

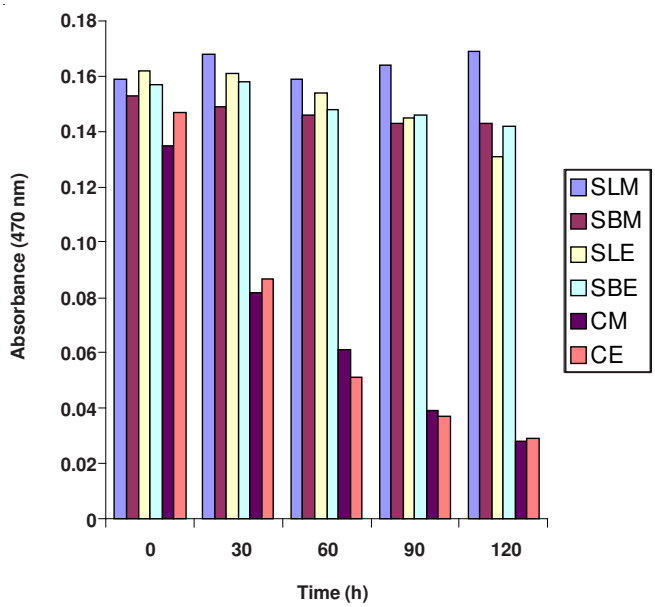

Fig. 1. Absorbance values of SBM, SLM, SBE, SLE extracts determined with β-carotene-linoleic acid model system. S-Sternbergia, SBM-bulb-methanol, SBE-bulb-ethanol, SLM-leaf-methanol, SLE-leaf-ethanol, CM-control-metanol, CE-control-etanol

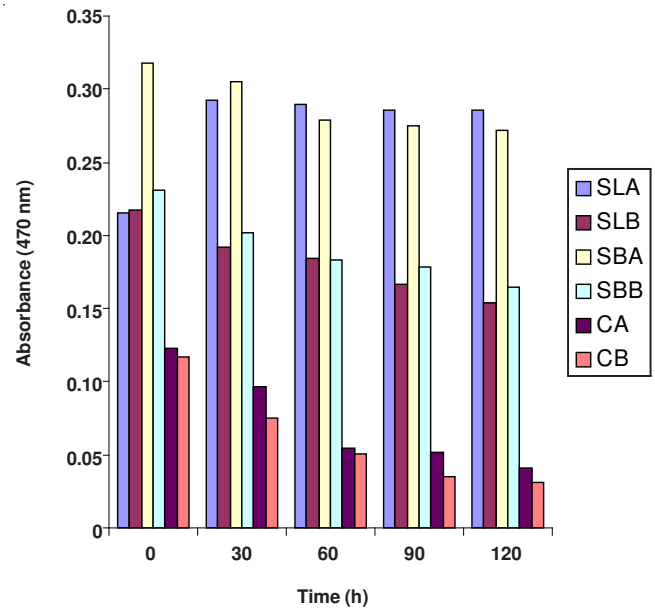

Fig. 2. Absorbance values of SBA, SLA, SBB, SLB extracts determined with β-carotene-linoleic acid model system. S-Sternbergia, SBA-bulb-acetone, SBB-bulb-benzene, SLA-leaf-acetone, SLB-leaf-benzene, CA-control-asetone, CB-control-benzene

The antioxidant activity efficiency were also calculated and given in Fig. 3. As it can be seen from this figure, the highest antioxidant activity efficiency is determined in extract SBE (91.85 ± 0.7 %) and the least efficiency in extract SBB (14.91 ± 0.3 %). Bulbs and leaves extracts were generally more potent than the free radical scavenging activity (Fig. 4).

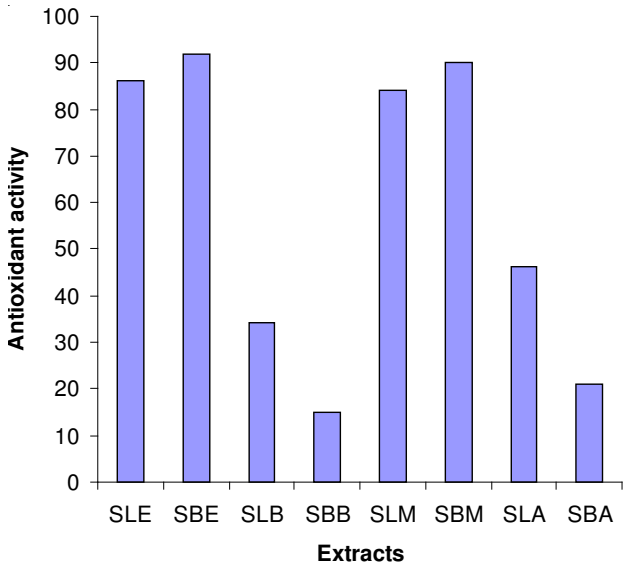

Fig. 3. Antioxidant activities efficiency in the methanol, ethanol, acetone and benzene extracts of *S. clusiana*. S-Sternbergia, SBM-bulb-methanol, SBE-bulb-ethanol, SBA-bulb-acetone, SBB-bulb-benzene, SLM-leaf-methanol, SLE-leaf-ethanol, SLA-leaf-acetone, SLB-leaf-benzene

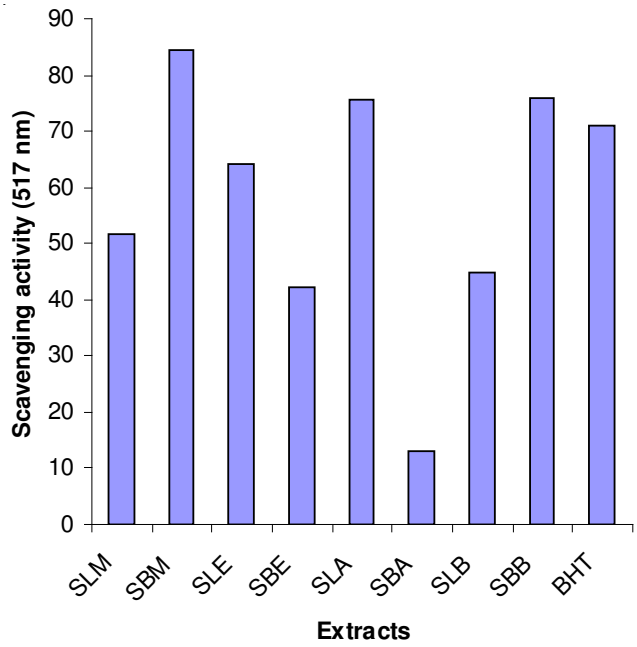

Fig. 4. Free radical scavenging capacity of the extracts with methanol, ethanol, acetone and benzene through DPPH method. S-Sternbergia, SBM-bulb-methanol, SBE-bulb-ethanol, SBA-bulb-acetone, SBB-bulb-benzene, SLM-leaf-methanol, SLE-leaf-ethanol, SLA-leaf-acetone, SLB-leaf-benzene, BHT-butylated hydroxytoluene

The antimicrobial activities of different extracts tested by agar dilution method were shown in Table-3. Inhibition zones of extracts were measured between 3 and 15 mm (Table-3).

TABLE-3
ANTIMICROBIAL ACTIVITIES OF THE AMPICILLIN, PENICILLIN, SLM, SBM, SLE, SBE, SLA, SBA, SLB AND SBB (INHIBITION ZONE DIAMETER, mm)

Microorganisms	Antibiotic and plant extracts								
			A	P	SBM	SBE	SBA	SBB	SLM	SLE
Escherichia coli ATCC 35218	18	nt	–	11 ± 2	–	9 ± 1	–	–	8 ± 1	7 ± 2
Staphylococcus aureus ATCC 25923	nt	31	4 ± 1	8 ± 1	7 ± 1	6 ± 0	–	7 ± 0	–	8 ± 0
Staphylococcus aureus ATCC 12598	nt	nt	–	5 ± 2	–	7 ± 0	–	6 ± 0	7 ± 1	6 ± 1
Pseudomonas aeruginosa NRRL B-23	nt	nt	6 ± 2	–	11 ± 1	3 ± 1	6 ± 0	–	3 ± 1	7 ± 2
Bacillus subtilis ATCC 6633	nt	2	–	7 ± 0	8 ± 2	5 ± 1	–	11 ± 2	–	–
Bacillus cereus RSKK 863	nt	22	3 ± 1	–	9 ± 1	10 ± 2	7 ± 1	3 ± 1	8 ± 1	–
Candida albicans ATCC 10239	nt	nt	8 ± 2	9 ± 1	15 ± 2	6 ± 1	–	9 ± 2	6 ± 1	8 ± 0

A: Ampicillin (10 mg), P: Penicillin (10 U), nt: Not tested; (–): No inhibition, S: Sternbergia, SBM: Bulb-methanol, SBE: Bulb-ethanol, SBA: Bulb-acetone, SBB: Bulb-benzene, SLM: Leaf-methanol, SLE: Leaf-ethanol, SLA: Leaf-acetone, SLB: Leaf-benzene.

Determination of total phenolic content: *S. clusiana* extracts containing the most phenolic component amount is the SBM extract containing the lowest phenolic component amount is the SBB extract. Phenolic component of SBE extract amount is partly high while the phenolic component amount of the other examples follow each other by showing a decrease (Table-2). Thereby it is possible to align the extracts according to their phenolic component amounts as in the following SBM > SBE > SBA > SLA > SLM > SLE > SLB > SBB. These were number of hydroxylic groups in phenolic compounds, so as their spatial orientation are proportional to molar response of this method[25].

Results of total antioxidant activity: The antioxidant activity were found to be decreasing in SBE, SBM, SLE, SLM, SLA, SLB and SBB extracts, respectively. The reason of the same plant's extracts showing different antioxidant activity may be due to the polarities of the solvents.

β-Carotene-linoleic acid model system depends on the principle that β-carotene discolours rapidly when no antioxidant is present as a result of the process in which free radicals produce hydroperoxides from linoleic acid. The absorbance value of the control were significantly lower (pCM < 0:08; pCE < 0:08.5; pCA < 0:1.4; pCB < 0:1, 3) than the plant extracts. All the extracts showed similar trends with a significant decrease of absorbance value until 1 h. This indicated that the samples, had acted as effective antioxidants in the β-carotene-linoleic system, which inhibited the oxidation activity of β-carotene (Figs. 1 and 2). The addition of antioxidant containing extracts to the system enables peroxide products formed from linoleic acid to be neutralized with these antioxidants and thus β-carotene preserves its characteristic yellow colour. The effect of thermal treatment on antioxidant activity and phenolic content were also studied. In the last decade a number of publications have been published in which antioxidant capacity of plant material, so as antioxidant characteristics of phenol compounds are tested, through different methods[26-28]. Because of this it is difficult to compare final results, even though there are the same plant species. Among all the extracts, SBA showed the highest absorbance value followed by SLA, SLB, SBB, SLM, SBM, SLE, SBE.

Free radical-scavenging activity: The higher absorbance of the samples showed a higher antioxidant activity in this study. Salah *et al*.[29] and Frankel *et al*.[30] have demonstrated that the stability of radical-scavenging activity depends on the antioxidant composition. Similar results were also reported for the antioxidant activity of content of *Amaranthus* sp. by Amin *et al*.[31].

The extracts and the free radical scavenging effects of BHT were tested on DPPH, a stable free radical. The results of the free radical scavenging effects were calculated to be a concentration of the 50 % of which was scavenged by DPPH (IC_{50}). The low IC_{50} value shows the high antioxidant activity. For this reason, the amount of unused DPPH in the system was determined and the percentages of the amount used were measured. The overall results showed that the values that make the solvents certain were as follows; BHT (71.16 ± 0.4 %) (SBM- 84.42 ± 0.4 % > SBB- 75.92 ± 1.4 % > SLA-75.54 ± 1.8 % > SLE-64.26 ± 0.9 % > SLM-51.58 ± 0.5 % > SLB-44.87 ± 1.2 % > SBE-42.28 ± 0.7 % > SBA-13.21 ± 1.2 %). Bulbs and leaves extracts were generally more potent than the free radical scavenging activity (Fig. 4). All these result from the high amount of flavonoid derivatives in the structure of extracts.

Antimicrobial activity results: The extracts which were provided from the *S. clusiana*, was fairly efficacious on *C. albicans* ATCC 10239. We were not able to observe noteworthy antimicrobial activity for SLM extraction. It has been demonstrated that SBE and SBA extracts had powerful effect on some bacteria; SBE extract on *E. coli* ATCC 35218 (11 ± 2 mm diameter), SBA extract on *P. aeruginosa* NRRL B-23 (11 ± 1 mm diameter), SLE extract on *B. subtilis* ATCC 6633 (11 ± 2 mm diameter) and SBB on *B. cereus* RSKK 863 (10 ± 2 mm diameter).

SBE, SBA, SLE and SBB extracts have showed mostly effect on Gr(+) bacteria. SBE and SBA extracts have showed mostly effect on Gr(–) bacteria. Extracts of *S. clusiana* are quite effective on *C. albicans* ATCC 10239 except for SLM. In this results showed that extracts of S.clusiana have revealed antioxidant, free-radical scaving and antimicrobial activity.

REFERENCES

1. D.L. Mahdavi, S.S. Deshpande and D.K. Salunkhe, Food Antioxidants: Toxicological Aspects of Food Antioxidant, Marcel Dekker Press, New York (1995).
2. C.A. Rice-Evans, N.J. Miller and G. Paganda, *Free Radic. Biol. Med*., **20**, 933 (1996).
3. A. Moure, M. Jose, D.F. Cruz, J.M. Dominguez, J. Sineiro, H. Dominguez, M.J. Nünez and J.C. Parajo, *Food Chem*., **72**, 145 (2001).
4. J. Pokorny, *Trends Food Sci. Technol*., **2**, 223 (1991).
5. J. Kanner, E. Frankel, R. Granit, B. Germaan and J.E. Kinsella, *J. Agric. Food. Chem.*, **42**, 64 (1994).
6. T.B. Ng, F. Liu and Z.T. Wang, *Life Sci.*, **66**, 709 (2000).
7. N.S. Alzoreky and K. Nakahara, *Int. J. Food Microbiol*., **80**, 223 (2003).
8. Z.X. Li, X.H. Wang and M.M. Zhang, *Tradit. Chin. Drug Res. Clin. Pharmacol*., **16**, 103 (2005).
9. B.A. Dafni and E. Werkerf, *New Phytologist*, **91**, 571 (1982).

10. F. Ünal, D. Yüzbasioglu and H. Duman, *Hereditas*, **126**, 277 (1997).
11. A.O. Sawsan and I.A. Fattash, *J. Horticult. Sci. Biotechnol*., **80**, 399 (2005).
12. N. Unver, G.I. Kaya and H.T. Ozturk, *Fitoterapia*, **76**, 226 (2005).
13. O. Goktas, R. Mammadov and M.E. Duru, *Afr. J. Biotechnol.*, **6**, 982 (2007).
14. G.S. Citoglu, B.S. Yilmaz and O. Bahadir, *Chem. Nat. Comp*., **44**, 826 (2008).
15. B. Gurbuz, N. Arslan, K.M. Khawar, A. Ipek, E.O. Sarihan, S. Ozcan, I. Parmaksiz and S. Mirici, *Scient. Horticult.*, **123**, 99 (2009).
16. P.H. Davis, Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh (1984).
17. Y.S. Velioglu, G. Mazza, L. Gao and B.D. Oomah, *J. Agric. Food Chem*., **46**, 4113 (1998).
18. V.L. Singleton, R. Orthofer and R.M. Lamuela-Raventos, *Met. Enzymol*., **299**, 152 (1999).
19. I. Amin and S.H. Tan, *Malays. J. Nutr*., **8**, 167 (2002).
20. J.H. Lee, J.H. Park and J.S. Choi, *Arch. Pharm. Res*., **19**, 223 (1996).
21. M. Cuendet, K. Hostettman and O. Potterat, *Helv. Chim. Acta*, **80**, 1144 (1997).
22. T. Katsube, Y. Tsurunaga and M. Sugiyama, *Food Chem*., **113**, 964 (2009).
23. R.M. Darwish, T. Aburjai and S. Al-Khalil, *J. Ethnopharmocol*., **l79**, 359 (2002).
24. C.H. Collins, P.M. Lyne and J.M. Grange, Microbiological Methods, Oxford University Press, London (2004).
25. E. Frankel, A. Waterhouse and P.L. Teissedre, *J. Agric. Food Chem*., **43**, 890 (1995).
26. J.A. Vinson, Y. Hao, X. Su and L. Zubik, *J. Agric. Food Chem*., **46**, 3630 (1998).
27. C.C. Teow, V.D. Truong, R.F. McFeeters, R.L. Thompson, K.V. Pecota and G.C. Yencho, *Food Chem*., **103**, 829 (2007).
28. R.G.O. Rumbaoa, D.F. Cornago and I.M. Geronimo, *Food Chem*., **113**, 1133 (2009).
29. N. Salah, N.J. Miller and G. Paganga, *Arch. Biochem. Biophys*., **322**, 339 (1995).
30. E.N. Frankel, S.W. Huang and R. Aeschbach, *Agric. Food Chem*., **44**, 131 (1996).
31. I. Amin, Y. Norazaidah and K.I.E. Hainida, *Food Chem.*, **94**, 47 (2006).