Bulgarian Journal of Agricultural Science, 21 (No 2) 2015, 261–265
Agricultural Academy

THE DETERMINATION OF TOTAL ANTIOXIDANT ACTIVITY, PHENOLICS AND PIGMENTS DURING VEGETATIVE AND REPRODUCTIVE PERIODS OF *ONOSMA* SPECIES

A. SIVACI[1], R. BINZET[2*], S. DUMAN[3] and H. I. TEKE[4]
[1] *Adiyaman University, Art and Science Faculty, Department of Biology, Adiyaman, Turkey*
[2] *Mersin University, Art and Science Faculty, Department of Biology, 33343 Mersin, Turkey*
[3,4] *Adiyaman University, Graduate School of Sciences, Adiyaman, Turkey*

Abstract

SIVACI, A., R. BINZET, S. DUMAN and H. I. TEKE, 2015. The determination of total antioxidant activity, phenolics and pigments during vegetative and reproductive periods of *Onosma* species. *Bulg. J. Agric. Sci.*, 21: 261–265

This study was carried out in order to evaluate the changes in antioxidant activity, phenolic compounds and pigments during the vegetative (April) and reproductive (May) periods of *Onosma* species *(Onosma rascheyana* and *Onosma sericea*). The antioxidant activity in vegetative period was higher (lowest activity) than reproductive period in *O. sericea* and *O. rascheyana*. In vegetative period, total phenolic compounds of *O. rascheyana* and *O. sericea* were lower than reproductive period. Pigments (chlorophyll a, chlorophyll b and carotenoids) in reproductive period were higher than vegetative in both *O. sericea* and *O. rascheyana*.

Key words: Onosma, antioxidant activity, phenolics, photosynthetic pigments, physiological development stage

Introduction

Onosma L. genus belongs to the family Boraginaceae, order of Lamiales and subclass of Dicotyledoneae. *Onosma* genus consisting of over 150 species is typical of xeric habitats as perennial, usually suffruticose or biennial herbs having its greatest species diversity in southeast Europe and Asia Minor and is known to include several serpentine endemics, especially in the southern Balkans (Stevanovic et al., 2003). It comprises biennial or perennial, hispid herbs, with flowers in terminal cymes, calyx accrescent, stamens inserted at the middle of the corolla, and generally four nutlets flat at the base. Besides these characters the species are distinguished on the basis of indumentums composed of specific trichomes, called stellate setae, which led to several mistakes in taxonomy in the past (Ball, 1972). Anatolia is an important centre of origin for *Onosma* comprising about 102 species (108 taxa), 51 of which and 1 variety are endemic to Turkey (Riedl, 1978; Davis et al., 1988; Yıldırımlı, 2000; Riedl et al., 2005; Binzet and Orcan, 2007; Kandemir and Türkmen, 2010; Aytaç and Türkmen, 2011; Guner, 2012; Koyuncu et al., 2013). The genus has been divided into three sections: *Protonosma, Podonosma* and *Onosma*. *Protonosm*a and *Podonosma* sections are represented by one species, the other *Onosma* species belong to *Onosma* section. This section is separated into two subsections according solely to indumentum type: *Asterotricha* (Boiss.) Gürke, and *Haplotricha* (Boiss.) Gürke. *O. sericea* belong to *Haplotricha* subsection and local name is kağıt emcek, and *O. rascheyana* belongs to *Asterotricha* subsection and local name is van emcek (Güner, 2012).

Several *Onosma* species are used in folk medicine, herbs and dyes. In folk medicine, these plants are employed for burns, wounds and ailments (Khajuria and Jain, 1993; Ozgen et al., 2003). *O. sericea* Willd., *O. microcarpum* Steven ex DC and *O. argentatum* Hub.-Mor. are used for the treatment of wounds in rural areas in Turkey (Ozgen et al., 2003, 2004). The flowers of some species are consumed as vegetables (Oztürk and Ozçelik, 1991). Some *Onosma* species contain shikonin which is used for the treatment of hemorrhoids. These substances are also use-

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

ful in cosmetic industry (Koul and Sambyal, 1993). In northern Cyprus, boiled the dried leaves of *O. fruticosa* Sm. is used for the relief of respiratory tract disorders (Viney, 1994). Root extract is used for pneumonia and typhoid fever and also used for dyeing hairs (Khan and Khatoon, 2008). The dye is obtained by boiling the roots of *Onosma trachytricha* Boiss in Manavgat region, Antalya vil. (Bulut, 2006).

Most antioxidants isolated from higher plants are polyphenols, which show biological activity as antibacterial, anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating effects (Larson, 1988). Sources of natural antioxidants are generally plant phenolic substances (Atoui et al., 2005; Huang et al., 2005; Mathew and Abraham, 2006; Skerget et al., 2005).

A literature survey has shown that there is no report on the total antioxidant activity, phenolics and pigments of *Onosma* species. Thus we decided to investigate the total antioxidant activity, phenolic and pigments contents of *Onosma* species, namely *Onosma sericea* and *Onosma rascheyana* during the vegetative (April) and reproductive (May) periods.

Materials and Methods

Plant materials

The examined species were collected from C7 Adıyaman, Sincik, Sincik-Arıkonak 2–3 km, slopes, and open forest and roadside, 1400 m. Taxonomic descriptions of the specimens were made according to Riedl (1978).

Determination of DPPH free radical scavenging

Leaf samples collected from *O. sericea* and *O. rascheyana* were dried. Samples were taken to methanol (MeOH) and extracted by shaking in water bath for 3 hours. Methanol extracts were then evaporated under vacuum until they became dried. Color of 2,2-diphenyl-1-picrylhydrazyl (DPPH) changes in the presence of antioxidant in the medium. Fifty micro liters of various concentrations of *Onosma* species extracts were dissolved in methanol and added to in 5 mL of a 0.004% methanol solution of DPPH. The mixture was incubated at room temperature for 30 minutes and absorbance values were measured at 517 nm (Gulluce et al., 2003). Inhibition percent (*I*%) of DPPH was calculated according to the following equation:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

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. Inhibition is concentration dependent, and extract concentration providing 50% inhibition (IC_{50}) is calculated from the graph that plotted inhibition percentage against extract concentration. Analyses were repeated thrice.

Determinationof total phenolics

Leaf samples taken from *O. sericea* and *O. rascheyana* were homogenized in 2.5 ml ethanol and shaken in water bath at 25°C for 24 h. After filtration 1 ml ethanol, 5 ml distilled water and 1 ml Folin-Ciocalteu reagent were added to 1 ml of the filtered samples and shaken well. After 3 minutes, 3 ml of Na_2CO_3 (2%, w/v) was added and shaken in a dark medium at intervals of 2 hours. Absorbance values were read at 760 nm and amounts were determined according to standard gallic acid equivalence (Slinkard and Singleton, 1977; Chandler and Dodds, 1983). Analyses were repeated thrice.

Determination of Pigments

Extraction and purification of the pigments were made according to De Kok and Graham (1989). The leaf samples of *O. sericea* and *O. rascheyana* were taken into acetone and homogenized. The homogenized samples were centrifuged. The absorbance values of centrifuged samples were read at 662, 645 and 470 nm according to Lichtenthaler and Wellburn (1983) and were calculated. Analyses were repeated thrice.

Statistical Analysis

All analyses were performed in three replicates. SPSS version 15.0 was used for statistical analyses. To determine the differences between the means, student *t*-test with a 95% confidence was used. Differences at 5% ($P < 0.05$) level were considered as significant.

Results

Free radical scavenging-DPPH

It was determined that total antioxidant activity varied according to plant development stage and species (Figure 1).Total antioxidant capacity (IC_{50}) in the leaves of *O. sericea* and *O. rascheyana* was higher in vegetative period (low antioxidant activity) compared to their reproductive period (high antioxidant activity) ($P < 0.05$). IC_{50} value for *O. sericea* in vegetative period was 544 µg mL^{-1} and 274 µg mL^{-1} in reproductive period (Figure 1) ($P < 0.05$). IC_{50} value for *O. rascheyana* in vegetative period was 430 µg mL^{-1} and 326 µg mL^{-1} in reproductive period (Figure 1) ($P < 0.05$). Total antioxidant capacity of both species was different from each other.

Total phenolics

Phenolics in the leaves of *O. sericea* ve *O. rascheyana* in reproductive period were higher than their vegetative period (Figure 2) ($P < 0.05$). Total phenolic amount of *O. sericea* in vegetative period was 0.20 µg mg^{-1} and 0.48 µg mg^{-1} in reproductive period (Figure 2) ($P < 0.05$). Total phenolic amount of

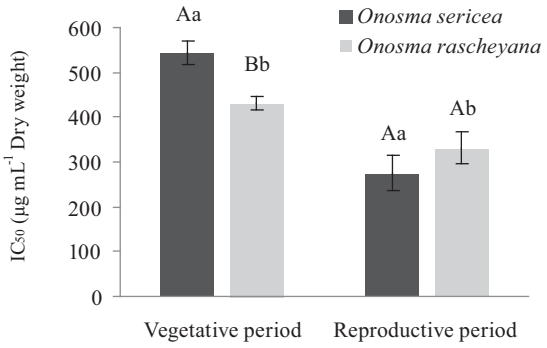

Fig. 1. IC_{50}values in leaves of *O. sericea* and *O. rascheyana* in DPPH
(It was found that the values shown with different letters are important in terms of statistics – P < 0.05, but the ones shown with the same letters are not.) (Small letters indicate the comparison between the periods and the capital letters indicate the comparison between the species)
(*t* test, confidence limit 95%)

O. rascheyana in vegetative period was 0.30 µg mg^{-1} and 0.42 µg mg^{-1}in reproductive period (Figure 2) (P < 0.05).

Pigment changes

The highest Chl a levels in both species were determined in reproductive period (Figure 3) (P < 0.05). In vegetative period, Chla levels of both species were found to be close to each other (Figure 3) (P > 0.05). Chla amounts for *O. sericea* in vegetative period were 0.47 mg g^{-1} and 0.79 mg g^{-1} in reproductive period, for *O. rascheyana* in vegetative period 0.46 mg g^{-1} and 0.65 mg g^{-1} in reproductive period.

It was found that Chl b levels in both species were the highest in reproductive period (Figure 4) (P < 0.05). In two periods, Chlb levels in both species were similar. Chlb amounts for *O. sericea* and *O. rascheyana* in vegetative period were 0.13 mg g^{-1} and 0.14 mg g^{-1} respectively. In repro-

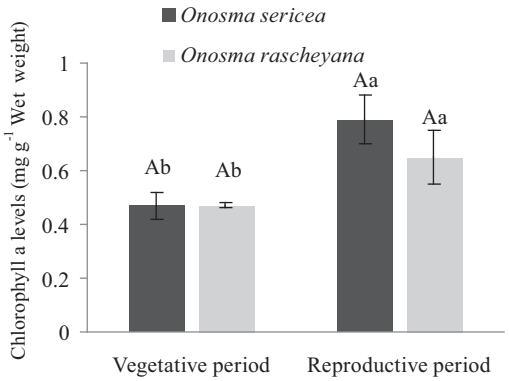

Fig. 3. The change of chlorophyll a contents in leaves of *O. sericea* and *O. rascheyana*
(It was found that the values shown with different letters are important in terms of statistics – P < 0.05, but the ones shown with the same letters are not.) (Small letters indicate the comparison between the periods and the capital letters indicate the comparison between the species)
(*t* test, confidence limit 95%)

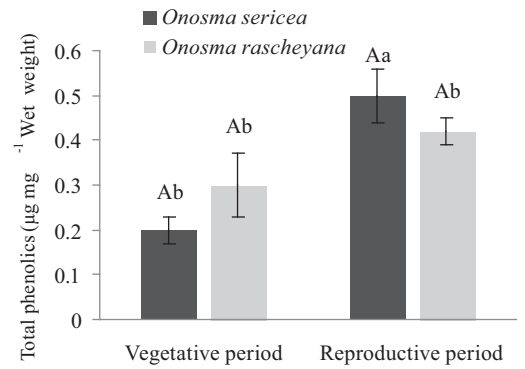

Fig. 2. The change of total phenolics in leaves of *O. sericea* and *O. rascheyana*
(It was found that the values shown with different letters are important in terms of statistics – P < 0.05, but the ones shown with the same letters are not.) (Small letters indicate the comparison between the periods and the capital letters indicate the comparison between the species)
(*t* test, confidence limit 95%)

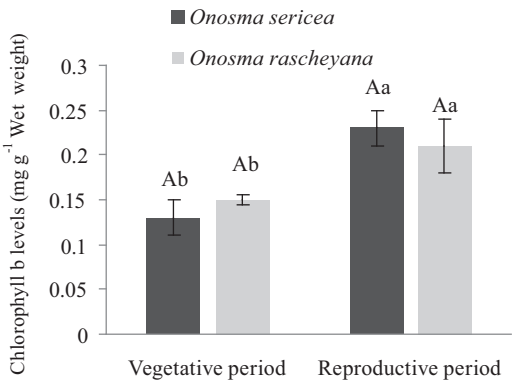

Fig. 4. The change of chlorophyll b contents in leaves of *O. sericea* and *O. rascheyana*
(It was found that the values shown with different letters are important in terms of statistics - P < 0.05, but the ones shown with the same letters are not.) (Small letters indicate the comparison between the periods and the capital letters indicate the comparison between the species)
(*t* test, confidence limit 95%)

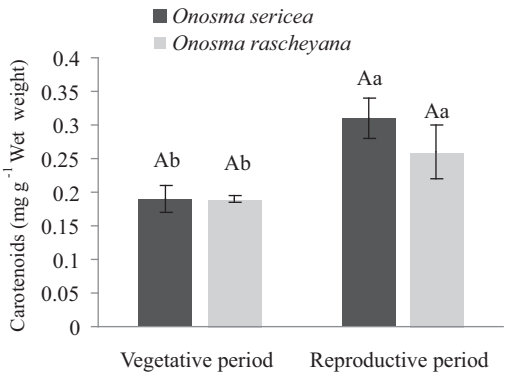

Fig. 5. The change of carotenoids in leaves of *O. sericea* and *O. rascheyana*
(It was found that the values shown with different letters are important in terms of statistics – P < 0.05, but the ones shown with the same letters are not.) (Small letters indicate the comparison between the periods and the capital letters indicate the comparison between the species)
(*t* test, confidence limit 95 %)

ductive period, Chlb levels were 0.23 mg g^{-1} for *O. sericea* and 0.20 mg g^{-1} for *O. rascheyana.*

Carotenoid levels of *O. sericea* and *O. rascheyana* were the highest in reproductive period as Chl a and Chl b changes (Figure 5) (P < 0.05). Carotenoid levels for *O. sericea* in vegetative period were 0.18 mg $g^{-1}$and 0.30 mg g^{-1} in reproductive period, for *O. rascheyana* in vegetative period – 0.18 mg g^{-1} and 0.25 mg g^{-1} in reproductive period.

Discussion

Many studies have stated that phenolics and antioxidant activity in plants change depending on biological and environmental factors (Rapisarda et al., 1999; Bâno et al., 2003; Moore et al., 2006). Baâtour et al. (2012) reported that total polyphenols, flavonoids, condensed tannins, phenolic content and DPPH of *Origanum majorana* L. changed according to salinity and development stage.

In the study conducted by Karray-Bouraoui et al. (2010) with *Mentha pulegium* L.*,* researchers found that antioxidant activity and phenolics changed depending on environment and development stage. Cirak et al. (2013) reported that there could be relationship between plant parts and developmental stages during the phenological cycle of the *Hypericum triquetrifolium* Turra and its chemical contents. In present study, antioxidant activity in *Onosma* species changed depending on physiological stages and species (Figure 1).

Medini et al. (2011) showed that phenolic content and antioxidant activity of *Limonium densiflorum* Kuntze were affected significantly depending on solvent, physiological development stage and their interactions. They found highest phenolic contents to be at the flowering stage (summer period). In this study, similar to reviewed studies above, phenolic compounds and antioxidant activity were found to be higher at flowering stage (reproductive) than vegetative stage (Figures 1 and 2). This result may be related to environmental conditions such that plants may be accumulating the phenolics under negative environmental conditions.

In carrots, during the transition from the vegetative phase to the reproductive stage there were no significant changes in the accumulation of photosynthetic pigments (Najafpour, 2012). Carotenoids play major a role in the protection of plants against photo oxidative damage and are part of the antioxidant defense system (Wılhelm and Helmut, 2003). The present study found that pigment levels (Chl a, Chl b and carotenoids) of *Onosma* species were the highest in reproductive period regarding antioxidant activity and phenolics (Figures 3, 4 and 5).

Conclusions

This study demonstrated that antioxidant activity, phenolics and pigment levels (Chla, Chlb and carotenoids) of *O. rascheyana* and *O. sericea* changed based on development stages of plants (vegetative and reproductive). The antioxidant activity, phenolics and pigment levels of *O. sericea* and *O. rascheyana* were the highest in reproductive period. *Onosma* species can be used in reproductive period for medicinal purposes.

Acknowledgments

This study was supported by Adıyaman University Scientific Research Projects Unit (SRP).

Authors' contribution

A. Sivaci carried out design of the study, analysis, acquisition of data and interpretation, drafting the manuscript. R. Binzet identification of the plant species, acquisition of data and interpretation, drafting the manuscript. S. Duman carried out analysis, acquisition of data and interpretation, statistical analysis. HİT carried out analysis, acquisition of data and interpretation.

References

Atoui, A. K., Mansouri, A., Boskou and G. P. Kefalas, 2005. Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chemistry*, **89:** 27–36.

Aytaç, Z. and Z. Türkmen, 2011. A new *Onosma* L. (Boraginaceae) species from southern Anatolia, Turkey. *Turkish Journal of Botany*, **35**: 269–274.

Baatour, O., I. Tarchoun, N. Nasri, R. Kaddour, J. Harrathi, E. Drawi, Mouhiba, Ben Nasri-Ayachi, B. Marzouk and M. Lachaâl, 2012. Effect of growth stages on phenolics content and antioxidant activities of shoots in sweet marjoram (*Origanum majorana* L.) varieties under salt stres. *African Journal of Biotechnology*, **11**: 16486–16493.

Ball, P. W., 1972. *Onosma* L. In: T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb (Eds). Flora Europea, vol 3. Diapensiaceae to Myoporaceae, *Cambridge University Press*, Cambridge, pp. 89–94.

Bâno, M. J., J. Lorente, J. Castillo, O. Benavente-Garcia, J. A. Rio, A. Ortuno, K. W. Quirin and D. Gerard, 2003. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems and roots of *Rosmarinus officinalis* antioxidant activity. *Journal of Agricultural and Food Chemistry*, **51**: 4247–4253.

Binzet, R. and N. Orcan, 2007. A new species of *Onosma* L. (Boraginaceae) From Southern Turkey. *Novon. A Journal for Botanical Nomenclature*, **17**: 8–10.

Bulut, Y., 2006. Useful Plants of Manavgat District (Antalya), Master Thessis, *Süleyman Demirel University*, Institute of Science, Isparta, 107 pp.

Chandler, S. F. and J. H. Dodds, 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell Reports*, **2**: 205–208.

Cirak, C., J. Radušienė, B. Karpavıcıene, N. Camaş and M. S. Odabaş, 2013. Changes in phenolic content of wild and greenhouse-grown *Hypericum triquetrifolium* during plant development. *Turkish Journal of Agriculture and Forestry*, **37**: 307–314.

Davis, P. H., R. R. Mill and K. Tan, 1988. Flora of Turkey and the East Aegean Islands. Vol **10**, *Edinburg University Press*, Edinburg, 590 pp.

De Kok, L. J. and M. Graham, 1989. Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of *Arabidopsis thaliano* during dark-induced and natural senescence. *Plant Physiology and Biochemistry*, **27**: 203–209.

Güllüce, M., M. Sökmen, D. Daferera, G. Agar, H. Ozkan, N. Kartal, M. Polıssıou, A. Sökmen, and F. Sahin, 2003. *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *Journal of Agricultural and Food Chemistry*, **51**: 3958–3965.

Güner, A. (ed.), 2012. A Checklist of the Flora of Turkey (Vascular Plants). Namaş Nurtan Ambalaj ve Matbaacılık San. *Ve Tic. A. Ş.*, Istanbul, 1290 pp.

Huang, D., B. Ou and R. L. Prior, 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, **53:** 1841–1856.

Kandemir, A. and Z. Türkmen, 2010. A new species of *Onosma* (Boraginaceae) from eastern Turkey. *Turkish Journal of Botany*, **34**: 277–282.

Karray-Bouraoui, N., R. Ksouri, H. Falleh, M. Rabhi, C. A. Jaleel, C. Grignon and M. Lachaâl, 2010. Effects of environment and development stage on phenolic content and antioxidant activities of *Mentha pulegium* L. *Journal of Food Biochemistry*, **34**: 79–89.

Khajuria, R. K. and S. M. Jain, 1993. Two new naphthoquinones from the roots of *Onosma hispidum*. *Indian Journal of Chemistry*, **32**: 390–391.

Khan, S. W. and S. Khatoon, 2008. Ethnobotanical studied on some useful herbs of Haramosh and Bugrote Vallies in Gilgit, Northern areas of Pakistan. *Pakistan Journal of Botany*, **40**: 43–58.

Koul, S. and M. Sambyal, 1993. Acetyl shikonin from callus cultures of *Onosma echioides* var. *hispidum. Fitoterapia*, **64**: 552–553.

Koyuncu, O., Ö. K. Yaylacı, Ö. Kurtuluş, O. Sezer and D. Öztürk, 2013. A new *Onosma* (Boraginaceae) species from Central Anatolia, Turkey. *Plant Systems Evolution*, **10**: 1839–1847.

Larson, R. A., 1988. The antioxidants of higher plants. *Phytochemistry*, **27**: 969–978.

Lichtenthaler, H. K. and A. R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls a and b of leaf exracts in different solvents. *Biochemical Society Transactions*, **11**: 591–592.

Mathew, S. and T. E. Abraham, 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chemistry*, **94**: 520–528.

Medini, F., R. Ksouri, H. Falleh, W. Megdiche, N. Trabelsi and C. Abdelly, 2011. Effects of physiological stage and solvent on polyphenol composition, antioxidant and antimicrobial activities of *Limonium densiflorum. Journal of Medicinal Plants Research*, **5**: 6719–6730.

Moore, J., J. G. Liu, K. Zhou and Y. Liangli, 2006. Effects of genotype and environment on the antioxidant properties of hard wheat-bran. *Journal of Agricultural and Food Chemistry*, **54**: 5313–5322.

Najafpour, M., 2012. *Applied Photosynthesis*, ISBN 978-953-51-0061-4, 422.

Ozgen, O., M. Coşkun, C. Kazaz and H. Seçen, 2004. Naphthoquinones from the roots of *Onosma argentatum* Hub.-Mor. (Boraginaceae). *Turkish Journal of Chemistry*, **28**: 451–454.

Ozgen, O., P. J. Houghton, Y. Ogundipe and M. Coşkun, 2003. Antioxidant and antimicrobial activities of *Onosma argentatum* and *Rubai peregrine. Fitoterapia*, **74**: 682–685.

Oztürk, M. and H. Özçelik, 1991. Useful Plants of East Anatolia. *SİSKAV*, Ankara, pp. 1-196.

Rapisarda, P., A. Tomaino, R. Locascio, F. Bonina, A. De Pasquale and A. Saija, 1999. Anti oxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry*, **47**: 4718–4723.

Riedl, H., 1978. Boraginaceae. In: P. H. Davis (Ed). Flora of Turkey and the East Aegean Islands, 6, *Edinburgh University press*, Edinburgh, pp. 237–437.

Riedl, H., R. Binzet and N. Orcan, 2005. A new species of *Onosma* (Boraginaceae-Lithospermeae) from southern Turkey. *Edinburgh Journal of Botany*, **61**: 127–130.

Skerget, M., P. Kotnik, M. Hadolin, A. R. Hras, M. Simonic and Z. Knez, 2005. Phenols, proanthocyanidins, flavones, and flavonol in some plant materials and their antioxidant activities. *Food Chemistry*, **89**: 191–198.

Slinkard, K. and V. L. Singleton, 1977. Total phenol analyses: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, **28**: 49–55.

Stevanovic, V., K. Tan and G. Iatrou, 2003. Distribution of endemic Balkan flora on serpentine. I. Obligate serpentine endemics. *Plant Systematics and Evolution*, **242**: 149–170.

Viney, D. E., 1994. An illustrated flora of North Cyprus. *Koeltz Scientific Books*, Koeningstein, pp. 6–447.

Wilhelm, S. and S. Helmut, 2003. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*, **24**: 345–351.

Yıldırımlı, Ş., 2000. The chorology of the Turkish species of Boraginaceae family. *The Herb Journal of Systemic Botany*, **7:** 257–272.

Received December, 2, 2014; accepted for printing April, 7, 2014.