Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

Ecological variability of the phenolic compounds of *Olea europaea* L. leaves from natural habitats and cultivated conditions

Milan Stanković, Svetlana Ćurčić, Nenad Zlatić & Biljana Bojović

To cite this article: Milan Stanković, Svetlana Ćurčić, Nenad Zlatić & Biljana Bojović (2017) Ecological variability of the phenolic compounds of *Olea europaea* L. leaves from natural habitats and cultivated conditions, Biotechnology & Biotechnological Equipment, 31:3, 499-504, DOI: 10.1080/13102818.2016.1275804

To link to this article: https://doi.org/10.1080/13102818.2016.1275804

BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT, 2017
VOL. 31, NO. 3, 499–504
http://dx.doi.org/10.1080/13102818.2016.1275804

Taylor & Francis
Taylor & Francis Group

ARTICLE; AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

OPEN ACCESS

Ecological variability of the phenolic compounds of *Olea europaea* L. leaves from natural habitats and cultivated conditions

Milan Stanković [a], Svetlana Ćurčić [b], Nenad Zlatić [a] and Biljana Bojović [a]

[a]Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Republic of Serbia; [b]Faculty of Education, University of Kragujevac, Jagodina, Republic of Serbia

ABSTRACT

Many compounds from the phenolic group, flavonoids in particular, are well-known antioxidants, although their role in plant response to stress is debatable. The aim of this study was to determine the variability of the phenolic content and the antioxidant activity of *Olea europaea* leaf samples from different habitats. The determination included measurement of the total quantity of phenolics, the flavonoid content, as well as the antioxidant activity of the two types of methanolic leaf extracts of *O. europaea* from several natural habitats in the Mediterranean region (Tunisia, Malta and Montenegro) and from cultivated conditions (France and Serbia). The results showed that both the total quantity of phenols and flavonoids as well as the intensity of antioxidant activity in the two types of extracts largely depended on the type of habitat. The total quantity of phenols and flavonoids was greater in the samples from cultivated plants which demonstrated the significance of certain conditions in terms of the correlation between the intensity of primary and secondary metabolism. However, the values of antioxidant activity in both types of extract were higher in the samples from natural habitats. The results showed that plants from natural habitats contain secondary metabolites with high biological activity. It could be speculated that these active substances play an important role in the adaptation of plants to the stress caused by arid conditions.

ARTICLE HISTORY
Received 4 October 2016
Accepted 19 December 2016

KEYWORD
Olea europaea; Mediterranean region; natural habitats; cultivated conditions; secondary metabolism

Introduction

The olive tree (*Olea europaea* L., family Oleaceae) is an evergreen tree or shrub cultivated for its fruits. Its height can reach between 8 and 15 m, mostly depending on the climate conditions. Initially, the fruits are of green colour and up to 2.5 cm long. However, with the process of maturation, fruits become dark purple in colour [1].

As the Mediterranean region is extremely dry, with high temperatures and therefore with high level of ultraviolet radiation, the plants of this climate, including the olive species, have specific mechanisms of adaptation to these abiotic conditions. Consequently, it is a widely accepted fact that the olive species are tolerant to drought and show remarkable persistence in shallow soils and soils of bad quality. This characteristic of olive trees has brought about great interest in their cultivation in arid areas [2].

Using some physiological, biochemical and morpho-anatomical mechanisms, olive trees reduce water loss and maintain the water content when periods of drought begin. These mechanisms also influence the ability of plants to tolerate dehydration when the drought intensifies [3].

It has been suggested that the lack of water causes lipid peroxidation, since two markers of oxidative damage, malondialdehyde content and lipoxygenase activity, increase in leaves and roots of olive species in drought periods. It has been, therefore, concluded that the higher the activities of some antioxidant enzymes and non-enzymatic antioxidants are, the better the plant protection against oxidative stress closely associated to drought impact is [4,5].

The factors that may influence the content, quality and quantity of these active substances mainly rest upon the type of plant habitat, the predominance of certain abiotic factors in the habitat, the season of the year as well as upon the presence or absence of unfavourable conditions in which the plant grows [6–9]. All plant organs contain flavonoids; however, their quantity varies depending on the plant species or ecological and genetic factors [10–15]. Bioactive compounds isolated from *O. europaea* leaves have antiviral, antimicrobial, antioxidant, anticancer, anti-inflammatory and hypoglycaemic properties [16–21].

CONTACT Milan Stanković mstankovic@kg.ac.rs

The aim of this study was to comparatively analyse the total phenolic content, flavonoid content and antioxidant activity of two types of methanolic extract of *O. europaea* leaves sampled in natural habitats located in the Mediterranean (Tunisia, Malta and Montenegro) and in cultivated conditions (France and Serbia). The purpose of this comparative analysis was to determine the variability of the phenolic content and the antioxidant activity of *O. europaea* leaf samples from different habitats.

Materials and methods

Chemicals

The Folin–Ciocalteu's reagent, aluminium chloride hexahydrate were from Fluka Chemie AG (Buchs, Switzerland). Gallic acid, rutin hydrate, 2,2-dyphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Methanol and sodium hydrogen carbonate were purchased from Zorka pharm (Šabac, Serbia).

Plant material

The samples of *O. europaea* leaves were collected in 2013 from Mediterranean natural habitats in Tunisia (Mornaguia, 36°47'21"N, 10°02'44"E), Malta (Mellieha, 35°57'36"N, 14°21'14"E) and Montenegro (Budva, 42°16'48"N, 18°47'45"E) with vegetation of sclerophyllous evergreen trees and shrubs as well as from cultivated conditions (pot planted, protected during winter period) in France (Tours, 47°23'04"N, 0°39'58"E) and Serbia (Kragujevac, 44°01'49"N, 20°54'42"E). Terminal branches from 3–5 representative individuals were sampled as plant material. The collected plant material was air-dried at ambient temperature in a dark environment. The dried leaves were stored in sealed containers until use.

Preparation of plant extracts

Samples of dried leaves of *O. europaea* were ground to obtain plant powder. For plant extract preparation (E type 1) powdered plant material (10 g) was extracted with 250 mL of methanol (98%). Thus extracted samples were filtrated with Whatman No. 1 filter paper after 48 h and subsequently evaporated. In order to determine the phenolic content and antioxidant activity in plant material (E type 2), each powdered sample was dissolved in methanol at a concentration of 1 mg/mL and filtered after 48 h.

Determination of total phenolics in the plant extracts

The total phenolics concentration was determined spectrophotometrically (ISKRA, MA9523-SPEKOL 211) [22]. The used concentration of methanol solution of the extract was 1 mg/mL. For the preparation of the reaction mixture, 0.5 mL of methanol solution of the plant extract, 2 mL of $NaHCO_3$ solution and 2.5 mL of Folin–Ciocalteu reagent were taken. The test samples were prepared in triplicate. Based upon the measured absorbance (λ_{max} = 765 nm), the total concentration of phenolics was expressed as gallic acid equivalents (mg of GA/g).

Determination of total flavonoids in the plant extracts

The concentration of flavonoids in the tested extracts was determined spectrophotometrically [23]. The test sample contained 1 mL of methanol solution of the extract at a concentration of 1 mg/mL and 1 mL of $AlCl_3$ solution dissolved in methanol. The test samples were incubated and the absorbance was measured at 415 nm. The test samples were prepared in triplicate for each analysis and the mean value of the absorbance was obtained. Based upon the measured absorbance, the concentration of flavonoids was expressed in terms of rutin equivalent (mg of Ru/g).

Evaluation of antioxidant activity

The efficiency of the plant extract to scavenge 1,1-dyphenyl-2- picrylhydrazyl (DPPH) free radicals *in vitro* was measured as described in [24,25]. The solution of plant extract was prepared in methanol to achieve a concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 μg/mL. Diluted solutions were mixed with prepared DPPH reagent. After incubation, the absorbance was measured at 517 nm. Antioxidant activity was expressed as the half-maximal inhibitory concentration (IC_{50} values in μg/mL). In the presented results, the antioxidant efficiency of the extract increased with the decreasing of IC_{50} values.

Data analysis

Experimental measurements were performed in triplicate and values are expressed as arithmetical means from three analyses with standard deviation (±SD). The data analysis was done using Origin (OriginLab, Northampton, MA, USA).

Results and discussion

Total phenolics content

The spectrophotometric method for measurement of the quantity of total phenolic compounds in the extracts and plant material of the species *O. europaea* using the Folin–Ciocalteu reagent is based on measurement of the redox potential of phenols in solution. Their dissolution produces a proton and a phenoxide anion which reduces the Folin–Ciocalteu reagent to ion generating blue colour. The values obtained for the total quantity of phenolic compounds are presented in Table 1.

The total quantity of phenols in the leaf extract of *O. europaea* (E type 1) was in the range of 127.18–314.69 mg of GA/g. In the extracts from the samples taken from natural *O. europaea* habitats, the quantity of total phenols varied between 127.18 and 250.17 mg of GA/g of extract. The highest concentration was detected in the sample from Tunisia (250.17 mg of GA/g), whereas the sample from Malta showed a much lower quantity (149.23 mg of GA/g). The lowest concentration was measured in the sample from Montenegro (127.18 mg of GA/g). Regarding the samples from cultivated plants, the greatest quantity of phenols was observed in the sample from France (314.69 mg of GA/g), followed by the sample from Serbia with somewhat lower concentration (249.31 mg of GA/g).

The total quantity of phenolic compounds in plant material (E type 2) ranged between 51.71 and 79.77 mg of GA/g of plant material. In the samples from natural habitats, the total quantity of phenols varied between 51.71 and 67.40 mg of GA/g of solution. The greatest quantity was measured in the Tunisian sample (67.40 mg of GA/g) followed by the sample from Malta (61.77 mg of GA/g) and that from Montenegro (51.71 mg of GA/g), which ranked third. Of the samples from cultivated conditions, the one from France (79.77 mg of GA/g) exceeded that from the Serbian locality in total quantity of phenolic compounds (71.29 mg of GA/g).

The observed higher content of total phenols in the plant extracts and plant material from cultivated conditions compared to those from natural habitats conditions could most likely be attributed to the fact that cultivated plants differ not only in geographical position, but also in terms of the conditions in which they grow. The presence and influence of abiotic factors such as planned sufficient intake of minerals and water in cultivated conditions signify the absence of stress in terms of mineral, water, light and temperature regime. Such conditions stimulate the primary metabolism of cultivated plants, which results in production of higher biomass and more intensive secondary metabolism. Differences in the secondary metabolites content and activity between plant samples from cultivated conditions and natural habitats have been previously reported in comparative studies [26]. The correlation between more intensive primary metabolism and secondary metabolism has been demonstrated in the case of fertilization of cultivated *Salvia officinalis* L. [27].

Table 1. Total quantity of phenolic compounds in the extracts (E type 1) and plant material (E type 2) of *O. europaea* samples.

Locality	Plant extract (E type 1)	Plant material (E type 2)
Tunisia	205.17 ± 0.04	67.40 ± 0.01
Malta	149.23 ± 0.11	61.77 ± 0.02
Montenegro	127.18 ± 0.03	51.71 ± 0.01
France	314.69 ± 0.12	79.77 ± 0.04
Serbia	249.31 ± 0.08	71.29 ± 0.03

Note: Values presented as equivalents of gallic acid, mg of GA/g of extract, i.e. plant material. Values are means (±SD) from three experiments.

Flavonoids content

Next, the concentration of flavonoids in the extracts was determined by a spectrophotometric method using $AlCl_3$ in the process of which metal complexes are produced (Table 2). The concentration of flavonoids in the leaf extracts of *O. europaea* (E type 1) varied between 52.40 and 129.39 mg of Ru/g of extract. In the samples from natural habitats, the concentration of flavonoids ranged from 52.40 to 119.79 mg of Ru/g of extract. The highest concentration was measured in the Tunisian sample (119.79 mg of Ru/g), whereas the sample from Malta showed a considerably lower concentration (55.85 mg of Ru/g). The lowest concentrations were observed in the sample from the locality of Montenegro (52.40 mg of Ru/g). In the samples from cultivated conditions, the concentration of flavonoids in the sample from the Serbian locality (129.39 mg of Ru/g) exceeded that from the French locality (103.77 mg of Ru/g).

The concentration of flavonoids in dry plant material (E type 2) ranged between 22.22 and 37.98 mg of Ru/g. The concentration of flavonoids in the samples collected from natural habitats varied from 22.22 to 25.79 mg of Ru/g of plant material. The highest concentration was measured in the sample from Malta (25.79 mg of Ru/g), whereas the samples from the localities in Tunisia (22.73 mg of Ru/g) and Montenegro (22.22 mg of Ru/g) showed lower concentrations. With regard to the samples from cultivated conditions, the greatest quantity of flavonoids was observed in the sample from Serbia (37.98 mg of Ru/g), followed by the samples from France (26.67 mg of Ru/g). The obtained values of the concentration of flavonoids in the plant material suggest smaller variability in comparison with the variability observed for the total quantity of phenols.

Overall, there was a similar trend in the dynamics of synthesis and accumulation of total phenol compounds

Table 2. Flavonoid concentration in the extracts (E type 1) and plant material (E type 2) in O. *europaea* samples.

Locality	Plant extract (E type 1)	Plant material (E type 2)
Tunisia	119.79 ± 0.08	22.73 ± 0.01
Malta	55.85 ± 0.02	25.79 ± 0.03
Montenegro	52.40 ± 0.04	22.22 ± 0.01
France	103.77 ± 0.05	26.67 ± 0.01
Serbia	129.39 ± 0.06	37.98 ± 0.02

Note: Values are presented as rutin equivalents, mg of Ru/g of extract, i.e. plant material. Values are means (±SD) from three experiments.

in the samples from both cultivated and non-cultivated olive trees. Differentiation between individuals from cultivated and natural conditions on the basis of the quantity of flavonoids has been shown in the analysis of samples of cultivated, non-cultivated and micropropagated *Cecropia glaziovi* Senth [11]. This comparison showed the significance of the conditions in the areas of cultivation with respect to correlation between the intensity of primary and secondary metabolism.

Antioxidant potential

The antioxidant activity (IC_{50}) of the extracts (E type 1) varied from 30.04 to 113.30 μg/mL (Table 3). Among the samples collected from natural habitats, the IC_{50} values ranged from 30.04 to 75.91 μg/mL, with the Tunisian samples showing the highest antioxidant activity (IC_{50} of 30.04 μg/mL). Lower antioxidant activity was observed in the samples from Malta (IC_{50} of 35.49 μg/mL) and Montenegro (IC_{50} of 75.91 μg/mL). Of the plants from cultivated areas, the Serbian sample showed higher potential to scavenge DPPH radicals (IC_{50} of 94.39 μg/mL) than the sample from France (IC_{50} of 113.30 μg/mL). The antioxidant activity of dry plant material (E type 2) was shown to range from 105.44 to 231.39 μg/mL. In the samples from natural habitats, the IC_{50} values varied between 105.44 and 231.39 μg/mL. The sample taken in Tunisia showed the highest DPPH-scavenging ability (105.44 μg/mL), whereas the samples from Malta (IC_{50} of 129.26 μg/mL) and Montenengro (IC_{50} of 231.39 μg/mL) showed significantly lower antioxidant activity. The sample from Serbia (IC_{50} of 160.71 μg/mL) was observed to have higher DPPH-scavenging ability than the sample from France (IC_{50} of 211.55 μg/mL).

Table 3. Antioxidant activity (IC_{50} values expressed as μg/mL) of the O. *europaea* extracts (E type 1) and plant material (E type 2).

Locality	Plant extract (E type 1)	Plant material (E type 2)
Tunisia	30.04 ± 0.92	105.44 ± 1.56
Malta	35.49 ± 0.85	129.26 ± 1.22
Montenegro	75.91 ± 0.77	231.39 ± 1.65
France	113.30 ± 1.12	211.55 ± 1.89
Serbia	94.39 ± 1.03	160.71 ± 1.23

Note: Values are means (±SD) from three experiments.

Comparative analysis

The analysis of the obtained results suggests variability with similar dynamics in the total quantity of phenols and flavonoids in the extracts of the samples from natural habitats. In terms of the quantity of secondary metabolites (total phenols and flavonoids in particular) in the leaves of olive trees growing in natural habitats, the three localities were ranked in decreasing order as follows: Tunisia, Malta and Montenegro. The variability may be explained with respect to the role of secondary metabolites in the process of plant adaptation to the ecological conditions in its habitats [8]. In terms of ecological conditions, the most notable characteristic that the sampled localities had in common was aridity. Taking into account the geographical location, aridity is a dominant ecological condition for most Mediterranean habitats. Generally speaking, aridity constitutes a complex of ecological factors (increased insolation, higher temperatures and water deficiency) which mutually exert influence in the habitat. The plant species that make up the vegetation in the Mediterranean habitats have developed morphological, anatomical, phenological and physiological adaptations. The stages of evergreen sclerophyllous vegetation alternate due to the intensity of aridity as well as to the influence of some other abiotic factors. Maquis, the habitat in Montenegro from which the *O. europaea* plant material was sampled, represents the first stage of degradation of typical Mediterranean forest vegetation, while garrigue, the type of habitat in Malta and Tunisia, stands for the next stage, which usually develops in habitats with greater aridity [28]. The association between the gradients of the ecological conditions in the habitats from which the plant material was collected and the antioxidant activity of the secondary metabolites suggests that their variability is due to their significance in the adaptation to abiotic stress. The plants sampled in this study originated from habitats influenced by drought. The response of plants to drought depends on the type of species, life form and the period of drought and includes a series of physiological, anatomical, morphological and phenological mechanisms.

Apart from changes in metabolism, growth and development, one of the principal effects of stress caused by drought is intense accumulation of reactive oxygen species as well as disturbed antioxidant–prooxidant balance in plant tissues, which puts the integration of vital biomolecules and biomembranes at risk. The role of increased antioxidant enzymatic or non-enzymatic defence is of particular importance in stress reduction. The main elements of the non-enzymatic defence are secondary metabolites, mostly from the group of

phenolic compounds [29]. Their significance lies in their increased synthesis and accumulation during drought stress and is corroborated on several levels in laboratory conditions. The results obtained in this study demonstrated the significance of the habitat type in terms of the antioxidant activity in extracts and plant material. The antioxidant activity in both types of extracts was higher in the plants sampled in natural habitats as opposed to the values measured in samples from cultivated plants. For example, correlation between the type of habitat and the intensity of antioxidant activity has been shown in *Prunella vulgaris* L. collected in natural and cultivated habitats [30]. The production of secondary metabolites and, consequently, of phenolic compounds increased due to the differences in the intensity of aridity in different habitats as well as due to the influence of unfavourable ecological factors on plants causing physiological stress in the affected species.

Since phenols contain hydroxyl groups which enable them to inhibit the activity of free radicals, the phenolic content of plants could be regarded as a direct influence on the antioxidant activity of plant extracts [31]. Overall, our observation that the samples from natural habitats exhibited greater antioxidant activity, regardless of the smaller amount of phenolic compounds as compared to the samples from the cultivated plants, suggests that not only the quantity but also the structure of phenolic compounds contributes to phenolic activities in terms of their synthesis as determined by environmental conditions. However, there was association between the concentration of phenols and the ROS-scavenging potential only in the samples from natural habitats. Increase in both production of secondary metabolites and the antioxidant activity points to the necessity to carry out further studies in order to evaluate their role in physiological adaptations to different abiotic factors.

Conclusions

The results from this study demonstrated variation in the phenolic content and the antioxidant activity of the studied *O. europaea* extracts from some natural habitats in the Mediterranean (Tunisia, Malta and Montenegro) and cultivated conditions (France and Serbia). Greater antioxidant activity was observed in the samples from natural habitats. It could be speculated that the observed variability indicates the role of the secondary metabolites in facilitating the adaptation of plants from arid areas to stress caused by higher temperatures and drought. The increased antioxidant activity of the extracts of plants from the natural habitats supports the suggestion that phenolics could be considered to play a key role in the ecophysiological adaptation of *O. europaea* to the specific ecological conditions predominant in the habitats from which the samples were collected. The obtained results could provide valuable guidance in the sustainable exploitation of plant material sampled from natural or cultivated habitats.

Acknowledgments

The authors acknowledge the contribution of Ivana Ćirković Miladinović (Faculty of Education, University of Kragujevac), Ana Vučićević (Faculty of Philology and Arts, University of Kragujevac) and Milica Novaković (Faculty of Science, University of Kragujevac).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Ministry of Science and Technological Development of the Republic of Serbia [grant number III41010].

ORCID

Milan Stanković http://orcid.org/0000-0001-9861-7700
Biljana Bojović http://orcid.org/0000-0002-7996-9652

References

[1] Haifa Group [Internet]. Nutritional recommendation for olives. Haifa: Haifa Chemicals Ltd; c2014 [cited 2016 Sep 11]. Available from: http://www.haifa-group.com/files/Guides/Olive_Booklet.pdf
[2] Bacelar EA, Santos DL, Moutinho-Pereira JM, et al. Immediate responses and adaptive strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci. 2006;170(3):596–605.
[3] Connor DJ, Ferreres E. The physiology of adaptation and yield expression in olive. In: Darnell R, Ferguson I, Hokanson SC, editors. Horticultural reviews. New Jersey (NJ): John Wiley & Sons; 2005. p. 155–229.
[4] Sofo A, Dichio B, Xiloyannis C, et al. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. Plant Sci. 2004;166:293–302.
[5] Bacelar EA, Santos DL, Moutinho-Pereira JM, et al. Physiological behaviour, oxidative damage and antioxidative protection of olive trees grown under different irrigation regimes. Plant Soil. 2007;292(1–2):1–12.
[6] Cheng A, Lou Y, Mao Y, et al. Plant terpenoids: biosynthesis and ecological functions. J Integr Plant Biol. 2007;49(2):179–186.
[7] Oh M, Trick HN, Rajashekar CB. Secondary metabolism and antioxidants are involved in environmental

adaptation and stress tolerance in lettuce. J Plant Physiol. 2009;166(2):180–191.
[8] Khan TA, Mazid M, Mohammad F. Status of secondary plant products under abiotic stress: an overview. J Stress Physiol Biochem. 2011;7(2):75–98.
[9] Karahan F, Avsar C, Ozyigit II, et al. Antimicrobial and antioxidant activities of medicinal plant *Glycyrrhiza glabra* var. *glandulifera* from different habitats. Biotechnol Biotechnol Equip. 2016;30(4):797–804.
[10] Chaves N, Escudero JC, Gutierrez-Merino C. Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* exudate. J Chem Ecol. 1997;23 (3):579–603.
[11] Luengas-Caicedo EP, Braga CF, Brandão CG, et al. Seasonal and intraspecific variation of flavonoids and proanthocyanidins in *Cecropia glaziovii* Sneth. leaves from native and cultivated specimens. Z Naturforschung C. 2007;62c:701–709.
[12] Stanković SM, Topuzović; M, Solujić; S, et al. Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. J Med Plant Res. 2010;4 (20):2092–2098.
[13] Stanković SM, Vassilev K, Stanković NM, et al. Inter-population variation in phenolic content of *Teucrium chamaedrys* L. from the localities in the Balkan Peninsula. Poster session presented at: Planta medica. 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research; 2011; Antalya.
[14] Stanković SM. Ecological study of *Teucrium montanum* L. – population, phenological and plant part variability of secondary metabolites concentration. Poster session presented at: International Botanical Congress: Botanikertagung; 2011; Berlin.
[15] Stanković SM, Nićiforović N, Mihailović V, et al. Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium* polium L. subsp. polium. Acta Soc Bot Pol. 2012;81(2):117–122.
[16] Micol V, Caturla N, Pérez-Fons L, et al. The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). Antiviral Res. 2005;66 (2–3):129–136.
[17] Silva S, Gomes L, Leitão F, et al. Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. Food Sci Tech Int. 2006;12(5):385–396.
[18] Pereira AP, Ferreira ICFR, Marcelino F, et al. Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. Cv. Cobrancosa) leaves. Molecules. 2007;12(5):1153–1162.
[19] Wainstein J, Ganz T, Boaz M, et al. Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. J Med Food. 2012;15(7):605–610.
[20] De Marino S, Festa C, Zollo F, et al. Antioxidant activity and chemical components as potential anticancer agents in the olive leaf (*Olea europaea* L. cv Leccino.) decoction. Anticancer Agents Med Chem. 2014;14(10):1376–1385.
[21] Tezcan G, Tunca B, Bekar A, et al. *Olea europaea* leaf extract improves the treatment response of GBM stem cells by modulating miRNA expression. Am J Cancer Res. 2014;4(5):572–590.
[22] Singleton VL, Orthofer R, Lamuela RRM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152–178.
[23] Quettier-Deleu C, Gressier B, Vasseur J, et al. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol. 2000;72(1–2):35–42.
[24] Takao T, Watanabe N, Yagi I, et al. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem. 1994;58(10):1780–1783.
[25] Kumarasamy Y, Byres M, Cox PJ, et al. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother Res. 2007;21(7):615–621.
[26] Conforti F, Statti G, Uzunov D, et al. Comparative chemical composition and antioxidant activities of wild and cultivated *Laurus nobilis* L. leaves and *Foeniculum vulgare* subsp. *piperitum* (Ucria) Coutinho seeds. Biol Pharm Bull. 2006;29(10):2056–2064.
[27] Nell M, Vötsch M, Vierheilig H, et al. Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). J Sci Food Agric. 2009;89:1090–1096.
[28] Globalbioclimatics.org [Internet]. Spain: Phytosociological Research Center; c1996–2015 [cited 2016 Sep 20]. Available from: http://www.globalbioclimatics.org/
[29] Karagöz A, Artun TF, Özcan G, et al. *In vitro* evaluation of antioxidant activity of some methanol extracts. Biotechnol Biotechnol Equip. 2015;29(6):1184–1189.
[30] Sárosi S, Bernáth J, Burchi G, et al. Effect of different plant origins and climatic conditions on the total phenolic content and total antioxidant capacity of self-heal (*Prunella vulgaris* L.). Acta Hortic. 2011;925:49–55.
[31] Tosun M, Ercisli S, Sengul M, et al. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. Biol Res. 2009;42(2):175–181.