**Journal of Applied Botany and Food Quality 86, 217 - 220 (2013), DOI:10.5073/JABFQ.2013.086.030**

Departamento de Química, Universidad de Las Palmas de Gran Canaria, Unidad Asociada al CSIC, Campus de Tafira, Las Palmas de Gran Canaria, Canary Islands, Spain

# Screening of the antioxidant properties of crude extracts of six selected plant species from the Canary Islands (Spain)

**Milagros Rico*, Idayra Sánchez, Cristina Trujillo, Norma Pérez**

(Received September 24, 2013)

## Summary

The extracts of six common plants from the Canary Islands were screened for their antioxidant activities and compared with several phenolic compounds of natural origin (quercetin, catechin, rutin and gentisic acid) and synthetic (butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)). The *in vitro* antioxidant activity determined by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method revealed that *Plantago major* L., *Artemisia canariensis* (Bess.) Lessing and *Bidens aurea* (Dryand.) Sherff exerted greater activity than the other plants (90.9%, 89.0% and 88.2% inhibition rate, respectively). The most active plants were *Bidens aurea* (Dryand.) Sherff and *Plantago major* L. (9.5 and 7.2 trolox µmol equivalents), when the cupric ion reducing antioxidant capacity assay (CUPRAC) was used. All the plants species exhibited higher antioxidant capacities than the synthetic antioxidants BHA and BHT. Among the natural phenolic compounds, gentisic acid was the most active. However, two of the plant extracts showed higher antioxidant activity than any other of the pure compounds studied, even than that of gentisic acid. The use of reversed phase high performance liquid chromatography (RP-HPLC) allowed the identification of the natural phenolic constituents listed above in *Bidens aurea* (Dryand.) Sherff and *Plantago major* L. extracts. Catechin and quercetin were the most prominent phenolic compounds. The presence of phenolic compounds in the plant extracts and their high antioxidant activities underline their phytomedicinal potentials. These plants may be exploited in the production of health foods and as an antioxidant carrier in the food and pharmaceutical industries.

## Introduction

The Canary Islands are an exceptional enclave in the world on account of their privileged climate. The temperature in the islands ranges from 17 º to 24 ºC all year round. The islands are volcanic and mountainous, and the varying altitudes create diverse habitats and ecosystems, making them very interesting from a botanical point of view. The high level of solar radiation forces plants to develop defence mechanisms against ultraviolet radiation through the accumulation of antioxidant substances. The following plant species grow anywhere and everywhere naturally: *Withania aristata* (Aiton) Pauquy (Solanaceae) (*W. aristata* P.), locally called orobal, are native of the Canary Islands, the Mediterranean and the Arabian region; *Plantago major* L., popularly called llantén, is a perennial medicinal herb that belongs to the highly diverse genus Plantago of the Plantaginaceae family; *Chenopodium ambrosioides* L. (*Ch. ambrosioides* L.), locally called pasote, is an herb of the Chenopodiaceae family, indigenous to South America, though it has been distributed to much of the world; *Bidens aurea* (Dryand.) Sherff (Asteraceae), called canary tea, is an european herb widely distributed in the Mediterranean areas and commonly used as digestive and sedative; *Forsskahlea angustifolia* Retz. (Urticaceae) (*F. angustifolia* R.), popularly known as ratonera, it is present in the seven Canary Islands; *Artemisia thuscula Cav.* (*Asteraceae*), a synonym of *Artemisia canariensis* (Bess.) Lessing, is an endemic Canary Islands species, widespread in all the islands in which different species of nitrophilous vegetation are dominant. It is most often found in coastal and mid zones of Western Canary Islands (100-350 m). These species were selected because they have been used by the Canary folklore medicine as panacea for a great diversity of health problems and have traditionally been consumed as infusion or herbal tea (Ortega et al., 2000; Samuelsen, 2000; Darias et al., 2001; Martín-Herrera et al., 2008). These plant species are estimated to be botanical medications in widespread domestic use among the population.

Plant polyphenols have been implicated in diverse functional roles, including plant resistance against microbial pathogens and animal herbivores such as insects (antibiotic and antifeeding actions), protection against solar radiation, besides reproduction, nutrition, and growth (Harborne and Williams, 2000). Phenolic compounds have also been reported to prevent diseases resulting from oxidative stress. They have the ability to counteract the damaging effects of free radicals in tissues and thus, they are believed to protect against cancer, atherosclerosis, heart diseases and several diseases (Bors et al., 1990; Dillard and German, 2000; Yao et al., 2004; Dai and Mumper, 2010; Ferrazano et al., 2011; Quideau et al., 2011). By other hand, the most widely food synthetic preservatives: butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been restricted because of serious concerns about their carcinogenic potential (Ito et al., 1986; Kahl and Kappus, 1993). Toxic effects of BHA and BHT often occur only after high dosage and long-term treatment. However, BHA induces in animals tumors of the forestomach, which are dose dependent, whereas BHT induces liver tumors in long-term experiments. Because there is no indication of genotoxicity of BHA and BHT, all published findings agree with the fact that BHA and BHT are tumor promoters. Therefore, in recent decades, there has been a great interest in finding new and safe antioxidants from natural sources to replace the synthetics used in food preservation. The chemical diversity of antioxidants makes it difficult to separate and quantify individual antioxidants from the vegetable matrix and the total antioxidant power is often more meaningful to evaluate health-beneficial effects because of the cooperative action of antioxidants (Gao et al., 2011). Recent studies revealed beneficial effects of plant extracts in ghee (butter oil) during accelerated oxidation in order to understand its potential use as an antioxidant in the food industries (Ghandi et al., 2013). The addition of mango seed kernel crude extracts (with high contents of phenolic compounds) at a level of 5% or above was more effective in prolonging the stability of buffalo ghee than the addition of BHA at the permitted level in ghee (0.02%) (Puravankara et al., 2000). By other hand, macaroni products incorporated with vegetal preparations exhibited improved antioxidant properties: the content of polyphenols increased from 0.46 to 1.80 mg per g of macaroni and the carotenoid content increased from 5 to 84 µg per g of macaroni, without affecting its cooking, textural and sensory properties (Ajila et al., 2010). The main objectives of this work consisted in the following, therefore: (1) to determine the antioxidant activities of

* Corresponding author

extracts derived from the plant species listed above with regard to their potential uses; (2) to compare the antioxidant activity of several pure phenolic compounds namely quercetin, catechin, rutin and gentisic acid (widely distributed in vegetables, fruits, tea, coffee, wine and other products (RABABAH et al., 2011; LÓPEZ et al., 2013; RICO et al., 2013) with those of the synthetic antioxidants BHT and BHA.

## Matateriales and methods

### Chemicals

Methanol (of HPLC grade) was obtained from Panreac (Barcelona, Spain) with formic acid, ammonium acetate, copper(II) chloride and 96% ethanol provided by Merck (Hohenbrunn, Germany) of analytical quality. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hidroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox) and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were from Sigma-Aldrich Chemie (Steinheim, Germany). Polyphenols quercetin and (+) catechin, were purchased from Sigma-Aldrich Chemie; rutin and gentisic acid were supplied by Merck (Hohenbrunn, Germany).

### Plant material

*W. aristata* P., *P. major* L., *Ch. ambroioide*s L., *B. aurea* S. and *F. angustifolia* R. were colleted at Rincon de Tenteniguada (977 m altitude, Valsequillo, Gran Canaria) in October, 2012. *A. canariensis* L. was collected in Tafira (320 m altitude, Gran Canaria) in October, 2012. Soon after collection, aerial parts of the plants were separated, shaken, weighed and frozen. The frozen samples were then freeze-dried and pulverized into powder using a blender (Moulinex, 600 W, Ecully Cedex, France) and were subsequently kept in the dark at -20 °C under nitrogen. The dried residues were weighed and the yield for water was calculated.

A voucher specimen has been deposited at the Herbarium of the Viera y Clavijo Botanical Garden in Las Palmas de Gran Canaria: *W. aristata* P. (LPA: 30929); *Plantago major* L. (LPA: 30930); *Ch. ambrosioides* L., (LPA: 30931); *Bidens aurea* (Dryand.) Sherff (Asteraceae) (LPA: 30932); *F. angustifolia* R. (LPA: 30933-30935), *Artemisia canariensis* (Bess.) Lessing (LPA: 30936).

### Preparation of the samples for DPPH and CUPRAC assays

Plant extracts: freeze-dried plant material (1.0 g) was extracted with methanol (18 mL) for 1 h at room temperature by mixing using a multipoint magnetic stirrer (ANM-10006, Paris, France). Each extract was filtered for removal of plant particles. After centrifugation at 3000 rpm for 10 min, the supernatant was collected and filtered through 0.45 mm filter paper and stored at 4 ºC.

Solutions of pure phenolic compounds: all the solutions (of the natural and synthetic pure phenolic compounds) were prepared at the concentration of 200 μg $mL^{-1}$.

### Free radical scavenging activity on DPPH

The reducing ability of the antioxidants on the DPPH radical was evaluated by measuring the loss of 1,1-diphenyl-2-picrylhydrazyl (DPPH) color at 515 nm after reaction with test extracts (BONDET et al., 1997). The sample solution (15 mL) was rapidly mixed with one mL of a solution of 0.1 mM DPPH. After 20 min incubation in darkness at ambient temperature (23 °C), the reduction of the DPPH radical was measured by monitoring the decline in absorbance (Abs) against a methanol blank at 515 nm using a Shimadzu 1700 UV-Vis spectrophotometer. The percentage inhibition was calculated by application of the equation: RSA = 100 (1 – Abs in the presence of sample/Abs in the absence of sample). The estimation of the RSA was carried out in triplicate, and the results were averaged. Values mean ± standard deviation of the three measurements.

### Cupric ion reducing antioxidant capacity (CUPRAC)

Then cupric ion reducing capacities were determined according to a previously reported method (APAK et al., 2004). A solution of $CuCl_2$ (125 μL $1.0x10^{-2}$ M), 125 μL of neocuproine ethanolic solution ($7.5x10^{-3}$ M) and 125 μL of $NH_4Ac$ buffer solution were added to a test tube, followed by mixing; 20 μL of sample followed by 605 μL of water were then added (total volume, 1 mL) and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. The estimation of the RSA was carried out in triplicate, and the results were averaged.

The same procedure was repeated for all standard trolox solutions (0.2-0.6 mM) and a standard curve was obtained with the following equation: Absorbance = 0.2859 x + 0.0762; $R^2$ = 0.9998. The results were expressed as trolox μmol equivalents (TR).

### Determination of the Phenolic Profile by RP-HPLC

The phenolic compounds catechin, gentisic acid, rutin and quercetin were quantified in line with a previously reported method (LÓPEZ et al., 2011). In brief, the elution conditions applied were 0-5 min, 20% B isocratic; 5-30 min, linear gradient from 20% to 60%B; 30-35 min, 60%B isocratic; 35-40 min, linear gradient from 60% to 20% B; and, finally, washing and reconditioning of the column. Each standard was individually tested to determine its retention times (RT) as follows: (+) catechin (RT: 12.7 min), gentisic acid (RT: 17.1 min), rutin (RT: 28.1 min) and quercetin (RT: 34.6 min) were well resolved. Simultaneous monitoring was set at 270 nm ((+)-catechin), 324 nm (gentisic acid) and 373 nm (rutin and quercetin) for quantification. Reproducibility, expressed as relative standard deviation (RSD), ranged from 1.91% to 5.81%. The accuracy was expressed as the recovery of standard compounds added to the pre-analyzed sample. The recovery was found to be in the range of 87.97%-115.79%. The limits of detection (LODs) were found to be in the range of 0.0003-0.1230 mg $mL^{-1}$ and the limits of quantification (LOQs) were observed in the range of 0.0008-0.4100 mg $mL^{-1}$.

## Results

### Evaluation of the water content

The content of water was as follows (Tab. 1): *B. aurea* S. > *P. major* L. > *Ch. ambroioides* L. > *W. aristata* P. > *A. canariensis* L. > *F. angustifolia* R.

**Tab. 1:** Content of water of the plant species (%).

| Plant species | Water content |
|---|---|
| *B. aurea* S. | 89.6 |
| *P. major* L. | 88.6 |
| *C. ambroioides* L. | 80.5 |
| *W. aristata* P. | 68.4 |
| *A. canariensis* L. | 62.7 |
| *F. angustifolia* R. | 43.2 |

### Free radical scavenging activity on DPPH

In Tab. 2 is shown the relative antioxidant efficiency of six plant extracts by quenching DPPH radical. Among the plants *P. major*

L., *A. canariensis* L. and *B. aurea* S. exerted more potent radical scavenging activity than any of the other samples (90.9%, 89.0% and 88.2% inhibition rate, respectively). The plant extract with the weakest scavenging potency was *Ch. ambrosoides* L. (29.1%), which had significantly lower activity than the other plant extracts. However, *Ch. ambrosoides* L. gave higher activity than BHA. Among the pure phenolic compounds of natural origin tested, gentisic acid showed the highest activity (67.8%), even much higher than those of the synthetic antioxidants BHA and BHT (22.7% and 5.0%, respectively) (Tab. 2).

**Tab. 2:** Radical scavenging activities of the samples expressed as inhibition percentage.

| Phenolic compound | RSA[a] | Plant species | RSA[a] |
|---|---|---|---|
| BHT | 5.0 ± 0.1 | *P. major* L. | 90.9 ± 0,5 |
| BHA | 22.7 ± 0.4 | *A. canariensis* L. | 89.0 ± 0,4 |
| Quercetin | 28 ± 2 | *B. aurea* S. | 88.2 ± 0.3 |
| Catequin | 21.3 ± 0.5 | *W. aristata* P. | 47 ± 3 |
| Rutin | 12 ± 1 | *F. angustifolia* R. | 45 ± 2 |
| Gentisic acid | 67.8 ± 0.9 | *Ch. ambrosoides* L. | 29.1 ± 0.4 |

[a] Values represented mean ± standard deviation of three measurements.

**Cupric ion reducing antioxidant capacity (CUPRAC)**

The CUPRAC assay was used to study the ability of the antioxidants in the extracts to reduce cupric copper to the cuprous form. When the reducing capacities (RC) of the plant extracts were compared, *B. aurea* S. and *P. major* L. showed the highest capacities (9.5 and 7.17 TR, respectively) (Tab. 3). The extract of *F. angustifolia* R. gave the weakest activity (1.49 TR). However, all the plant extracts exhibited higher antioxidant activities than those of the synthetic antioxidants TBA and TBH (1.26 and 0.09 TR, respectively) and natural quercetin, catechin and rutin (2.17, 0.96 and 0.62 TR, respectively). Gentisic acid showed the highest activity among the natural phenolic compounds (3.17 TR).

**Tab. 3:** Cupric ion reducing capability expressed as trolox µmol equivalents (TR).

| Phenolic compound | RC[a] | Plant species | RC[a] |
|---|---|---|---|
| TBH | 0.09 ± 0.06 | *B. aurea S.* | 9.5 ± 0.1 |
| TBA | 1.26 ± 0.02 | *P. major L.* | 7.17 ± 0.01 |
| Quercetin | 2.17 ± 0.00 | *A. canariensis* L. | 2.4 ± 0.1 |
| Catequin | 0.96 ± 0.00 | *Ch. ambrosoides* L. | 2.2 ± 0.2 |
| Rutin | 0.62 ± 0.00 | *W. aristata* P. | 1.88 ± 0.00 |
| Gentisic acid | 3.17 ± 0.02 | *F. angustifolia* R. | 1.49 ± 0.00 |

[a]Values represented mean ± standard deviation of three measurements.

**Determination of the Phenolic Profile by HPLC**

The proposed polyphenols quercetin, catechin, rutin and gentisic acid were identified in the extracts of the most active plants, *Plantago major* L. and *Bidens aurea* (Perkins) Sherff and the results are summarized in Tab. 4. Catechin and quercetin were the most abundant compounds of those under study.

**Tab. 4:** Polyphenol contents in plant extracts presented as average values ± standard deviation of two measurements in mg per gram of freeze-dried plant material.

| | *Plantago major* L. | *Bidens aurea* (Perkins) Sherff |
|---|---|---|
| Catechin | 1637 ± 79 | 1142 ± 24 |
| Gentisic acid | 97 ± 1 | 59 ± 3 |
| Rutin | 345 ± 30 | 287 ± 11 |
| Quercetin | 592 ± 32 | 473 ± 13 |
| Sum | 2671 | 1961 |

## Discussion

According to the existing food additive regulations, BHA and BHT are lawful for use individually or in combination at a maximum level of 0.02%, or 200 ppm, based on the lipid content of food products (Pratt, 1996; Reische et al., 1998). Therefore, we used the concentration 0.2 g $L^{-1}$ to test the antioxidant activity of the pure compounds. Because the extracts are complex mixtures that include active components at lower levels, they were prepared by solving 55.6 mg of freeze-dried plant material in 1 mL of methanol. Methanol was selected as extracting solvent because several studies have reported that high levels of phenolic compounds are associated with the use of polar solvents in the extraction (Hayouni et al., 2007; López et al., 2011). The *in vitro* antioxidant activities determined by using the DPPH and CUPRAC assays revealed that all the plant extracts exhibited much higher antioxidant activities than the synthetic phenolic compounds BHA and BHT, which are used as preservatives in many foods, cosmetic products and drugs (Tab. 2 and Tab. 3). By other hand, the most active phenolic compound of natural origin was gentisic acid (RSA: 67.8%; RC: 3.17 TR), which gave lower activity than those of the *B. aurea* S. and *P. major* L. extracts. Among the samples, the plant extracts exhibited the highest antioxidant activity compared to the individual standards probably due to a synergistic effect of combining the antioxidants (Jacobo-Velazquez and Cisneros-Zevallos, 2009).

Correlation was found between the activities of the pure compounds determined by both methods. However, there is no simple correlation between the radical scavenging activities and the copper reducing capacities of the plant extracts. This may be due to the fact that the CUPRAC method offers distinct advantages over other electron transfer based assays: applicability to both hydrophilic and lipophilic antioxidants (unlike DPPH); the redox reaction giving rise to a colored chelate of Cu(I)-neocuproine is relatively insensitive to a number of parameters adversely affecting DPPH, i.e., air, sunlight, humidity, and pH, to a certain extent. Moreover, the extracts are complex mixtures with active components that may vary the antioxidant potential or the responses to different kinds of assays. The lack of correlation is in agreement with other literature (Apak et al., 2007; Badarinath, 2010).

Quercetin, catechin, rutin and gentisic acid were identified in the extracts of *P. major* L. and *B. aurea* S., confirming their phytomedicinal potentials as natural sources of well-known antioxidant compounds (Silva et al. 2002).

## Conclusions

The results of this study confirmed that the selected plant materials, especially *P. major* L. and *B. aurea* S. gave higher antioxidant activities than those of the synthetic compounds BHA and BHT, commonly used as food preservatives. In addition, the presence

of well-known antioxidant compounds afford more than sufficient arguments for researching the viability of the use of the selected plants in the health and food industries in general, as well as in the pharmaceutical industry.

**Acknowledgments**
The authors would like to express their gratitude to María Ascención Viera Rodríguez for helping us to describe the plant species. Financial supports of the Caja Insular de Ahorros de Canarias are gratefully acknowledged.

## References

Ajila, C.M, Aalami, M., Leelavathi, K., Prasada Rao, U.J.S., 2010: Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. Innov. Food Sci. Emerg. Technol. 11, 219-224.

Apak, R., Guclu, K., Ozyurek, M., Karademir, S.E., 2004: Novel total antioxidant index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method, J. Agric. Food Chem. 52, 7970-7981.

Badarinath, A.V., RAo, K.M., Chetty, C.M.S., Ramkanth, S., Rajan, T.V.S., Gnanaprakash, K., 2010: A review on in-vitro antioxidant methods: comparisions, correlations and considerations. Int. J. PharmTech. Res. 2, 1276-1285.

Bondet, V., Brand-Williams, W., Berset, C., 1997: Kinetics and mechanisms of antioxidant activity using the DPPH free radical method, Lebensm. Wiss. Technol. 30, 609-615.

Bors, W., Hellers, W., Michel, C., Saran, M., 1990: Antioxidants in therapy and preventive medicine. New York, USA, Plenum press. 1, 165-170.

Dai, J., Mumper, R.J., 2010: Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 15, 7313-7352.

Darias, V., Martín-Herrera, D., Abdala, S. de la Fuente, D., 2001: Plants used in urinary pathologies in the Canary Islands. Pharm. Biol. 39, 170-180.

Dillard, C.J., German, J.B., 2000: Phytochemicals: Nutraceuticals and human health. J. Sci. Food Agric. 80, 1744-1756.

Ferrazzano, G.F., Amato, I., Ingenito, A., Zarrelli, A., Pinto, G., Pollio, A., 2011: Plant polyphenols and their anti-cariogenic properties: A review. Molecules. 16, 1486-1507.

Gandhi, K., Arora, S., Pawar, N., Kumar, A., 2013: Effect of Vidarikand (Extracts) on oxidative stability of Ghee: A comparative study, research and reviews. J. Dairy Sci. Technol. 1, 2319-3409.

Gao, Y.N., Liu, B.Y., Xu, D., Zhou, Q.H., Hu, C.Y., Ge, F.J., Zhang, L.P., Wu, Z.B., 2011: Phenolic compounds exuded from two submerged freshwater macrophytes and their allelopathic effects on *Microcystis aeruginosa*. Pol. J. Environ. Stud. 20, 1153-1159.

Harborne, J.B., Williams, C.A., 2000: Advances in flavonoid research since 1992. Phytochem. 55, 481-504.

Hayouni, E.A., Abedrabba, M., Bouix, M., Hamdi, M., 2007: The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 105, 267-273.

Ito, N., Hirose, M., Tsuda, H., Shirai, T., Tatematsu, M., 1986: Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem. Toxicol. 24, 1071-1082.

Jacobo-Velazquez, D.A., Cisneros-Zevallos, L., 2009: Correlations of antioxidant activity against phenolic content revisited: A new approach in data analysis for food and medicinal plants. J. Food Sci. 74, 107-113.

Kahl, R., Kappus, H., 1993: Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z. Lebensm. Unters Forsch. 196, 329-338.

López, A., Rico, M., Rivero, A., Suarez de Tangil, M., 2011: The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. Food Chem. 125, 1104-1111.

López, A., Suárez de Tangil, M., Vega-Orellan, O., Ramirez, A.S., Rico, M., 2013: Phenolic constituents, antioxidant and preliminary antimycoplasmic activities of leaf skin and flowers of Aloe vera (L.) Burn. F. (syn. A. barbadensis Mill.) from the Canary Islands (Spain). Molecules. 18, 4942-4954.

Martín-Herrera, D., Abdala, S., Benjumea, D., Gutiérrez-Luis, J., 2008: Diuretic activity of some *Withania aristata* Ait. fractions. J. Ethnopharmacol. 117, 496-499.

Ortega, C.A., María, A.O.M., Gianello, J.C., 2000: Chemical components and biological activity of *Bidens Subalternans*, *B. Aurea* (Astereaceae) and *Zuccagnia Puntacta* (Fabaceae). Molecules. 5, 465-467.

Pratt, D.E., 1996: Bailey's industrial oil and fat products. New York, USA, JohnWiley & Sons, Inc. 3, 524-545.

Puravankara, D., Boghra, V., Sharma, R.S., 2000: Effect of antioxidant principles isolated from mango (*Mangifera indica* L.) seed kernels on oxidative stability of buffalo ghee (butter-fat). J. Sci. Food Agric. 80, 522-526.

Quideau, S., Deffieux, D., Douat-Casassus, C., Pouységu, L., 2011: Plant polyphenols: Chemical properties, biological activities, and synthesis. Angew. Chem. Int. Ed. 50, 586-621.

Rababah, T.M., Ereifej, K.I., Esoh, R.B., Al-u'datt, M.H., Alrababah, M.A., Yang, W., 2011: Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. Nat. Prod. Res. *25*, 596-605.

Reische, D.W., Lillard, D.A., Eitenmiller, R.R., 1998. Food Lipids: chemistry, nutrition and biotechnology. Marcel Dekker, New York, USA, Akoh CC & Min DB eds., Inc., 423-448.

Rico, M., López, A., Santana-Casiano, J.M., González, A.G., González-Dávila, M., 2013: Variability of the phenolic profile in *Phaeodactylum tricornutum* diatom growing under copper and iron stress. Limnol. Oceanogr. 58, 144-152.

Samuelsen, A.B., 2000: The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. J. Ethnopharmacol. 71, 1-21.

Silva, M.M., Santos, M.R., Caroco, G., Rocha, R., Justino, G., Mira, L., 2002: Structure-antioxidant activity relationship of flavonoids: A re-examination. Free Radic. Res. 36, 1219-1227.

Yao, L.H., Jiang, Y.M., Shi, J., Barbera, S., Toma, F.A., Datta, N.N., Singanusong, R., Chen, S.S., 2004: Flavonoids in food and their health benefits. Plant Food Hum. Nutr. 59, 113-122.

Address of the corresponding author:
E-mail: mrico@dqui.ulpgc.es