The Annals of the University *Dunarea de Jos* of Galati
Fascicle VI – Food Technology, 34(1)

ORIGINAL ARTICLE

STUDIES ON TOTAL POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS FROM SELECTED LAMIACEAE SPECIES*

MARIA CIOROI, DANIELA DUMITRIU

Department of Chemistry,University Dunarea de Jos of Galati, Faculty of Siences,
Domneasca street, 47, 800008, Galati, Romania
maria.cioroi@ugal.ro

Received 9 April 2009
Revised 16 May 2009

Certain phytochemicals in species are attracting increased attention because of a wide range of biological activities especially the possible cancer preventive properties. Polyphenols, the natural antioxidants are present in plant extracts and they play a key role in antioxidative defence mechanisms in biological systems and they act as free radicals scavenging agents. Polyphenols might therefore inhibit development of coronary heart disease and cancers. Basil, oregano and sage are highly fragrant plants whose leaves are used as a seasoning herb for many different types of foods. Aqueous extracts were prepared from basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.) and sage (*Salvia officinalis* L.). To check the phenols presence, the UV-VIS spectrum was made.
The amount of polyphenolic compounds from selected Lamiaceae species was determined by spectrophotometry method using the Folin - Ciocalteau reagent and gallic acid as standard. The range of polyphenols total was between 516,352 mg/100g dried species and 859,617 mg/100g dried species. Reducing power has been established by measuring the redox potential of aqueous extracts. Antioxidant activity was directly correlated with the total amount of polyphenols in the species extracts.
The free reducing sugars in aqueous extracts from species were analyzed and correlated to the total content of polyphenols.

Keywords: Salvia officials, basil, oregano, antioxidant activity, Folin-Ciocalteau reagent, redox potential, Shaffer-Somogy method.

1. Introduction

Culinary herbs have been reported to possess antioxidant activities (Yanishlieva, Marinova, & Pokorny, 2006) suggesting that they might have potential human health benefits.
Salvia officials (called sage) is a popular plant belonging to the family of Labiatae and native to the Mediterranean region. The name "Salvia" comes from the Latin word meaning "the heal," which sums up the folkloric belief of its therapeutic properties for almost all kinds of aliments and its popularity in traditional medicine (Kasimu, 1998; Gulllen 1996).
Basil (family Lamiaceae) is a popular herb in the US and in the Mediterranean diets. The importance of Basil as a culinary herb, its historic usage, essential oil composition and phenolics have been well reviewed by Kintzios (2004).
Oregano is an important plant widely used in the South European cuisine and is a very popular herb in the Mediterranean countries. The leaves, dried herbs as well as the volatile oil of this aromatic perennial herb have been used for medicinal purposes for centuries. The positive effects of oregano on human health have now been attributed to its antioxidant activity both in the essential oil and soluble phenolic fractions (Eguchi, 1996; Engleberger, 1988; Peak, 1991). Kikuzaki and Nakatani (1988) isolated five different phenolic compounds from the methanol extract of leaves of oregano and among these rosmarinic acid was found to be present in the highest concentrations.

The objectives of this study were:

- to determine the total of polyphenols found in the aerial portions of the basil, oregano and salvia officinalis plants;

* Paper presented at the International Symposium *Euro - aliment 2009*, 9th – 10th of October 2009, Galati – ROMANIA

- to establish the oxidability of aqueous extracts from plants;
- to quantify the free reducing sugars in plant extracts.

2. Material and method

2.1. Plant materials

Common basil, oregano and sage were purchased from a local market in Galati.

2.2. Aqueous extracts preparation

The air parts of the analyzed plants, specially dried leaves (0.5 grams) were treated with double-distilled water at 100 degrees Celsius, for a period of 15 minutes. The samples were filtered. The resultant filtrates were made off up to 50 ml with double-distilled water. Three samples for each analyzed condiment plant were done.

2.3. Analysis of the total phenolic compounds in plant aqueous extracts

The amount of total phenolic content (TP) was determined as described by Waterhouse (2002).
Sample extracts were prepared by diluting 1:10. To proceed with the Folin-Ciocalteu method, 0.5 mL of sample extract followed by the addition of 0.25 mL of Folin-Ciocalteu reagent (2.0 N) and 2.5 mL sodium carbonate solution 0.1 M. The blank was prepared using the same chemical reagents excluding the extract.
The flasks were mixed well and left in the dark, at room temperature (25^{o}C) for 60 minutes, then the absorbance was read at λ = 750 nm (Mahnaz, 2009). UV/VIS spectrophotometer Secoman S750i and 1 cm quartz cells were used for all absorbance measurements.

2.4. Redox potential

In order to measure pH values and redox potential of samples the Multiparameter Consort C862 was used.

2.5. Free reducing sugars

The free reducing sugars from oregano, sage and basil extracts were measured using Shaffer-Somogyi method (Ney, 2009). Shaffer-Somogyi reagent interacts with free reducing sugars provided from the hydrolyzed glycosides of condiment extracts.

2.6. Statistical analysis

Statistical for Windows version XP was used for t-test calculations of correlation coefficient (r). It was used to assess the relationship between the antioxidative activity obtained via volumetric method and redox potential obtained by electrochemical measurements. The results of all experiments were expressed as the mean ±standard deviation upon three independent trials.

3. Results and discussions

Water extracts of sage, basil and oregano were analyzed in order to assess the amount of total polyphenols, redox potential and content in free reducing sugars of extracts.

3.1. The total content of polyphenolic

The total amount of polyphenolic compounds (PT) in water extracts was analyzed by the Folin–Ciocalteu method. The amount of PT was expressed as mg gallic acid/100g dried plants using the linear equation of the standard calibration curve: $Y = 0.153 \cdot X$.
The gallic acid was selected as standard due to its presence in almost all plants.
The UV-VIS spectrum of gallic acid was shown in figure 1A. The absorbance range of gallic acid is 250-350 nm. UV-VIS spectra of oregano, sage and basil extracts researches were done too. UV-VIS spectra of the samples were recorded with Spectro UV-VIS Double Beam PC 8 Scanning auto cell UVD-3200, Lobomed, INC Spectrophotometer. In figure 1B is shown UV-VIS spectrum of basil extract. In the range 280-340 nm was noticed a maximum of absorption as a shoulder which corresponds to gallic acid.
The total amount of phenolic compounds in the spices ranged from 5.1635 to 8.596 mg gallic acid/g dried plant and they come in the following order oregano> sage>basil (Figure 2). The results are

comparable to those obtained by Miliauskas *et al*. (2003), who studied some culinary plants and obtained ranges of 4.30 to 37.90 mg gallic acid/g. Wagensteen *et al*. (2004) obtained 19 mg gallic acid/g total phenolic compounds in some coriander plants. Ismail *et al*. (2004) detected ranges of 11.07 to 71.67 mg gallic acid/g in selected vegetables.

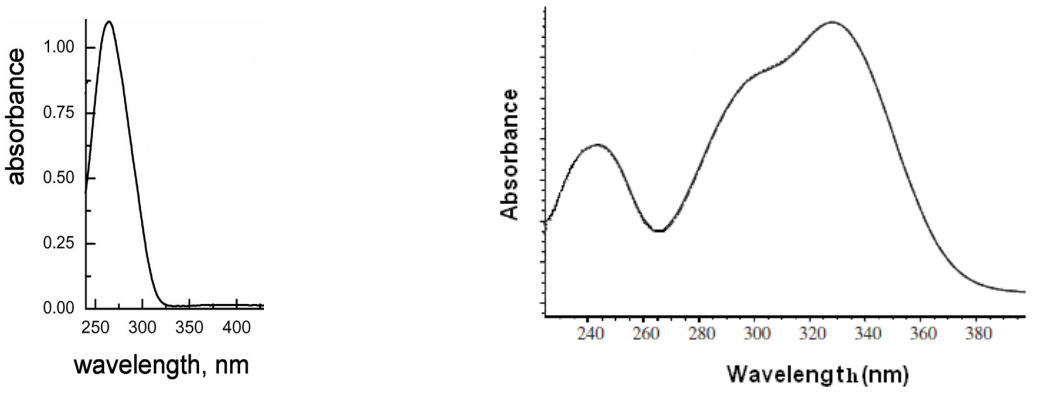

Figure. 1. UV–VIS spectra of Gallic acid (A) and of aqueous extract from basil leave (B)

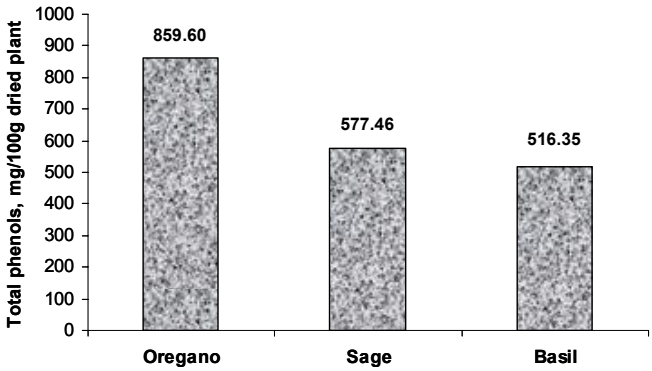

Figure 2. The total amount of polyphenols on oregano, sage and basil aqueous extracts

Capecka *et al*. (2003) detected between 11.07 and 14.06 mg gallic acid/g of the total phenolic compound contents in some herbs. Variation of phenolic compounds content arises due to several factors, which include the area of cultivation and other environmental stresses (Makkar, 1999).
The results regarding the amount of total polyphenols obtained by Folin Ceocalteu method could be influenced also by interference of other compounds found in plants (Hussain, 2008; Olah, 2003).

3.2. Antioxidant activity

A way of expression of the antioxidant capacity is to determine the reducing power of samples.
The measurement of pH and redox potential, E, of extracts was done by multimeter Consort C862. With the values obtained for pH and E values of rH were calculated which are presented in Table 1.

Table 1. The values of PT, E(mV), pH and rH parameters of aqueous extracts from condiments

Plants	PT (ppm)	E(mV)	pH	rH
Oregano	859.6	10.26	6.83	14.01687
Sage	577.46	18.5	6.21	13.06348
Basil	516.35	22	5.91	12.58522

The reducing character of samples could be correlated to the rH parameter. As shown in Table 1, oregano extracts achieve the reducing character with a more pronounced effect than the rest of spices followed by basil and sage extracts. Our results regarding the reducing character of condiment aqueous extracts are in agreement with the data provided by the scientific literature (Guveller, 1994; Guveller, 1996)

We found a good correlation between amount of total polyphenols and reducing character of assessed extracts, the correlation coefficient being 97.25% (Figure 3).

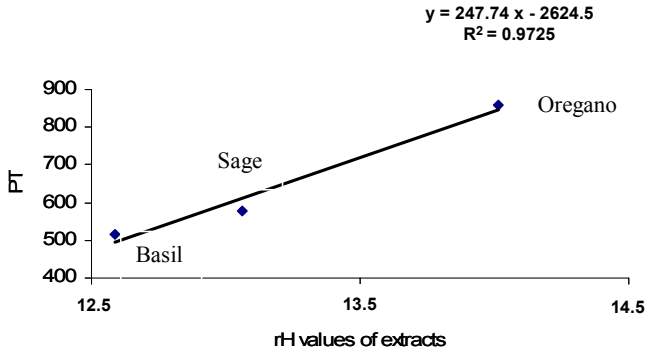

Figure 3. The correlation between PT and rH of aqueous extracts of condiments

3.3. Free reducing sugars

Antioxidant capacity of condiment extracts is due to the occurred polyphenols, ascorbic acid (Muchuweti, 2007), the content of ionic transitional metals but also to the content of free reducing sugars.

To assess the content of free reducing sugars which have an important role in the antioxidant character of condiment extracts was used the Shaffer-Somogy method.

The results of the reducing character were expressed as milliequivalents glucose/grams of plant in Table 2.

Tabel 2. Reducing character of analyzed condiments, mEg glucose/gram dried plant

Nr. crt.	Plants	mEg glucose/gram dried plant
1	Oregano	1.452
2	Sage	1.324
3	Basil	1.211

The free reducing sugars were expressed as equivalent of glucose which was used as standard in Shaffer- Somogy method. The highest content of reducing sugars occurs in oregano extracts followed by sage and basil. Results of our work are in agreement with literature data (Cervato, 2000). Reducing free sugars appear into extracts as a result of glycosides hydrolysis that takes place by treatment of plants with water at 100°C. By hydrolysis the glycosides evolve aglicons and sugars. In the most cases the aglicons are represented in plants by the polyphenols.

4. Conclusions

The general objective of this study was to determine the content of the total amount of polyphenols and the antioxidant character in plant extracts.

From the experimental data obtained result the following observations:

1. The content of polyphenols is higher in oregano extract rather than in sage and basil extracts.

2. The antioxidant activity expressed as rH increases from basil, sage to oregano aqueous extracts. The analyzed condiments show a real potential in providing the antioxidants for prevention of chronic diseases.
3. There is a correlation between the total amount of polyphenols, and the content of free reducing sugars.

The high content of the total amount of polyphenols in analyzed spices represents a characteristic parameter regarding their nutritional-physiological role as bioactive substances in human nutrition.
Further investigations are proposed to evaluate the relative composition of individual polyphenolic compounds from oregano, sage and basil.

References

Capecka, E., Marecczek, A., and Leja, M. 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chemistry*, **93**, 223-226.

Cervato, C., Carabelli, M., Gervasio, S., Cittera, A., Cazzola R. and Cestaro B. 2000. Antioxidant properties of Oregano[Origanum vulgare] leaf extracts. *Journal of Food Biochemistry*, **24**, 453–65.

Eguchi, Y., Curtis, O.F., Shetty, K. 1996. Interaction of hyperhydricity-prevent Pseudomonas sp. with oregano [Origanum *vulgare*] and selection of high phenolics and rosmarinic acid-producing lines. *Food Biotechnology*, **10**(3), 191–202.

Engleberger, W., Hadding, V., Etschenber, E., Graf, E., Leyck, S., Windelmann, J., and Parhnam, M.J. 1988. Rosmarinic acid. A new inhibitor of complement C3-convertase with anti-inflammatory activity. *International Journal of Immunopharmacology*, **10**, 729–37.

Gulllen, M.D., Cabo, N., and Burillo, J. 1996. Characterization of the essential oils of some cultivated aromatic plants of Industrial Interest. *Journal of the Science of Food and Agriculture*, **70**, 359-363.

Guveller, M.E., Richard, H., and Berst, C. 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extract of sage and rosemary. *Journal of the American Chemical Society*, **73**, 645-652.

Guveller, M.E., Brest, C., and Richard, H. 1994. Antioxidant constituants in sage (Salvia officialis). *Journal of Agricultural and Food Chemistry*, **42**, 665-669.

Hussain, A. I., Anwar, F., Sherazi, S. T. H., and Przybylski, R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. *Food Chemistry*, **108**, 986–995.

Ismail, A., Marjan, Z.M., and Foong, C.W. 2004. Total antioxidant activity and phenolic contents in selected vegetables. *Food Chemistry*, **87**, 581-586.

Kasimu, R., Tanak, K., Tezuka, Y., Gong, Z.N., Basmet, P., Namba T., Kadota, S. 1998. Comparative study of seventeen Salvia plants: Aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. *Chemical & Pharmaceutical Bulletin*, **46**, 500-504.

Kikuzaki, H., Nakatani, N. 1988. Structure of a new antioxidative phenolic acid from oregano (Origanum vulgare L.). *Agricultural Biological Chemistry*, **53**, 519–24.

Kintzios, S., Kollias, H., Straitouris, E., Makri, O. 2004. Scale-up micropropagation of sweet basil (Ocimum basilicum L.) in an airlift bioreactor and accumulation of rosmarinic acid. *Biotechnology Letters*, **26**(6), 521-523.

Khanavi, M., Hajimahmoodi, M., Cheraghi-Niroomand, M., Kargar, Z., Ajani, Y., Hadjiakhoondi, A., Oveisi, M.R. 2009. Comparison of the antioxidant activity and total phenolic contents in some *Stachys* species. *African Journal of Biotechnology*, **8**(6), 1143-1147.

Makkar, H.P.S. 1999. Quantification of tannins in tree foliage: A laboratory manual for FAO/IAEA. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, p. 1-29.

Miliauskas, G., Venskutonis, P.R., Van Beek, T.A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, **85,** 231-237.

Muchuweti, M., Kativu, E., Mupure, C.H., Chidewe, C., Ndhlala A.R., and Benhura, M.A.N. 2007. Phenolic Composition and Antioxidant Properties of Some Spices, *American Journal of Food Technology*, **5**, 414-420.

Ney, L. F., West, E. S. 2009. A potentiometric adaptation of the Shaffer - Hartmann sugar method, *The Journal of Biological Chemistry,***114** (2), 547-550

Olah, N., Radu, L., Mogosan, C., Hanganu, D., & Gocan, S. 2003. Phytochemical and pharmacological studies on Orthosiphon stamineus Benth. (Lamiaceae) hydroalcoholic extracts. *Journal of Pharmaceutical and Biomedical Analysis*, **33**, 117–123.

Peak, P.W., Pussel, B.A., Martyn, P., Timmermans, V., Charlesworth, J.A. 1991. The inhibitory effect of rosmarinic acid on complement involves the C5 convertase. *International Journal Immunopharmacology*, **13**, 853–857.

Yanishlieva, N. V., Marinova, E., & Pokorny, J. 2006. Natural antioxidants from herbs and spices. *European Journal of Lipid Science and Technology*, **108**, 776–793.

Wagensteen, H., Samuelsen, B.A., Malterude, E.K., 2004. Antioxidant activity in extracts from coriander. *Food Chemistry*, **88**, 293-297.

Waterhouse, A. L. 2001. "Determination of total phenolics", Handbook of food analytical chemistry, Unit I 1**.**1: Polyphenolics: p. 464–465, Wiley, New York.