

## Study on total polyphenols and reducing power of aqueous extracts from selected Lamiaceae species

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### Abstract

The Certain phytochemicals such as polyphenols found in culinary herbs are attracting increased interest due to a wide range of biological activities, especially their possible cancer preventive properties. Acting as free radicals scavenging agents, polyphenols play a key role in antioxidative defence mechanisms in biological systems. Polyphenols might therefore inhibit development of coronary heart disease and cancers. The amount of polyphenols in aqueous extracts from basil, sage and oregano was studied. Using the spectrophotometry method with the Folin - Ciocalteu reagent and gallic acid as standard, it was established that the range of total polyphenols in selected herbs was between 516,352 mg/100g and 859,617 mg/100g dried plant.

By measuring the redox potential of aqueous extracts, it was found the following variation of their reducing power: oregano> sage>basil. The free reducing sugars in the aqueous extracts were determined using the Shaffer- Somogy method.

**Keywords:** Sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.), oregano (*Origanum vulgare* L.), polyphenols, antioxidant activity, Folin - Ciocalteu reagent, UV-VIS spectrophotometry, redox potential, Shaffer-Somogy method

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### 1. Introduction

Culinary herbs have been reported to possess antioxidant activities (Yanishlieva, Marinova, & Pokorny, 2006) suggesting that they might have potential human health benefits.

Sage (*Salvia officinalis* L.) is native to the Mediterranean region. The latin name "Salvia" means "to heal," which sums up the folkloric belief of its therapeutic properties for almost all kinds of ailments and its popularity in traditional medicine (Kasimu, 1998, Gullen 1996). Basil (*Ocimum basilicum* L.) is a popular herb in the US and Mediterranean diets. The importance of basil as a culinary herb, its historic usage, essential oil composition and phenolic compounds have been well reviewed by Kintzios (2004).

Oregano (*Origanum vulgare* L.) is widely used in the South European cuisine, being very popular in the Mediterranean countries.

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 highest concentrations. In the present study, the total polyphenols content, the antioxidant activity and the free reducing sugars were measured on the aqueous extracts from basil, oregano and sage.

### 2. Material and methods

**2.1. Plants and materials.** Common basil, oregano and sage were purchased from a local market in Galati. All solvents/chemicals used were of analytical grade. Double-distilled water was used for the preparation of solutions and aqueous extracts.

**2.2. Aqueous extracts preparation.** The aerial parts of the analyzed plants, specially dried leaves (0.5 grams) were treated with double-distilled water at 100°C, for a period of 15 minutes. The samples were filtered. The resultant filtrates were made up to 50 mL with double-distilled water into a volumetric flask. Three samples for each analyzed plant were done.

**2.3. Analysis of total polyphenolic compounds in plant aqueous extracts.** Total polyphenolics (TP) were 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°C) for 60 minutes, then the absorbance was read at  $\lambda = 750$  nm (Mahnaz, 2009). UV/VIS spectrophotometer Secoman S750i and 1 cm quartz cells were used for all absorbance measurements. Total polyphenolics were quantified by calibration curve obtained from measuring the absorbance of known concentrations of gallic acid standard (25 to 150 g/mL solution). The concentrations are expressed as mg of gallic acid equivalents per 100 g of dry weight.

**2.4. Redox potential and pH values.** In order to measure the redox potential and pH values of sample extracts the Multiparameter Consort C862 was used.

**2.5. Free reducing sugars.** The free reducing sugars from oregano, sage and basil aqueous extracts were measured using the Shaffer-Somogyi method (Ney, 2009).

The Shaffer-Somogyi reagent interacts with the free reducing sugars provided from the hydrolyzed glycosides of herb extracts.

**2.6. Statistical analysis.** Statistical analysis for Windows version XP was used for t-test calculations of correlation coefficient (r). It was used to assess the relationship between the total polyphenolic compounds and reducing potential determined by electrochemical measurements.

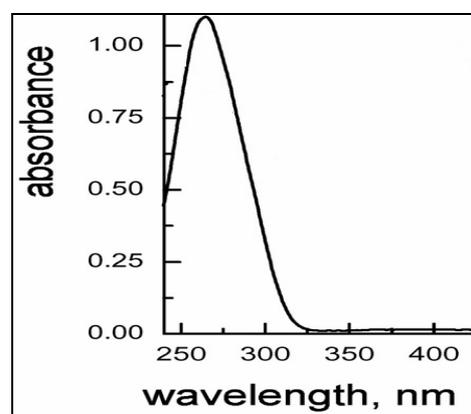
The results of all experiments were expressed as the mean  $\pm$  standard deviation upon three independent trials.

### 3. Results and discussion

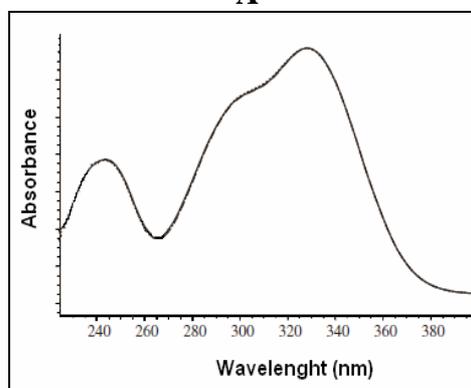
Aqueous extracts of sage, basil and oregano were analyzed in order to assess the amount of total polyphenols, redox potential and content of free reducing sugars.

**3.1. Total polyphenolic compounds.** The content of total polyphenolics (PT) in aqueous extracts was analyzed using 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 is shown in figure 1A. The absorbance range of gallic acid is 250-350 nm. UV-VIS spectra of oregano, sage and basil extracts 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. UV-VIS spectrum of basil extract is shown in figure 1B. In the range 280-340 nm it was noticed a maximum of absorption as a shoulder which corresponds to gallic acid.

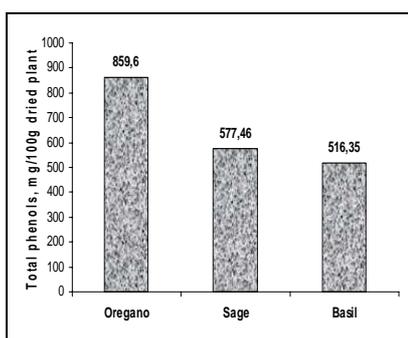


A



B

**Figure 1.** UV-VIS spectra of gallic acid (A) and of aqueous extract from basil leaves (B)



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

Capecka *et al.* (2003) detected between 11.07 and 14.06 mg gallic acid g<sup>-1</sup> total phenolic compound content 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 occurred in plants (Hussain, 2008, Olah, 2003).

**3.2. Antioxidant activity.** A way of expressing 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, rH values were calculated (table 1).

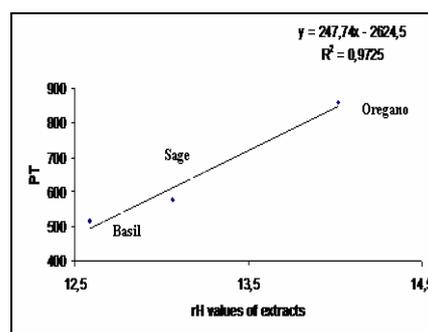
**Table 1.** The values of PT, E(mV), pH and rH parameters of aqueous extracts from selected culinary herbs

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 have a higher reducing character followed by basil and sage extracts.

The results regarding the reducing character of aqueous extracts in the present work are in agreement with data from literature (Guveller, 1994, Guveller, 1996).

A good correlation between the amount of total polyphenols and reducing character of assessed extracts was found, the correlation coefficient being 97.25% (figure 3).



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

**3.3. Free reducing sugars.** The antioxidant capacity of aqueous extracts is due to occurred polyphenols, ascorbic acid (M. Muchuweti *et al.*, 2007), content of ionic transitional metals and also content of free reducing sugars. The Shaffer-Somogy method was used to assess the content of free reducing sugars, which have an important role in the antioxidant character of aqueous extracts. The free reducing sugars were expressed as equivalent of glucose used as standard in the above method. The highest content of reducing sugars occurs in oregano extracts followed by sage and basil (table 2). Results of this work are in agreement with literature data (Cervato, 2000).

**Table 2.** Content of free reducing sugars as mE<sub>g</sub> glucose/g dried plant

Nr. crt.	Aqueous extracts	mE <sub>g</sub> glucose/g dried plant
1	Oregano	1.452
2	Sage	1.324
3	Basil	1.211

Reducing free sugars appear in aqueous extracts as a result of glycosides hydrolysis by treating the plants with water at 100°C. During hydrolysis the glycosides release aglicons and sugars. In most cases the aglicons are represented in plants by polyphenols.

#### 4. Conclusions

This paper presents data on the content of total polyphenols and antioxidant character in aqueous extracts of selected culinary plants. From the experimental data obtained the following conclusions may be drawn:

1. The content of total polyphenols is higher in oregano aqueous extracts than in sage and basil aqueous extracts.
2. The antioxidant activity expressed as rH increases from basil, sage to oregano aqueous

extracts. The analyzed herbs are a considerable source of antioxidants (polyphenols and also free reducing sugars).

3. There is a good correlation between the amount of total polyphenols, the reducing power and the content of free reducing sugars in aqueous extracts from selected culinary herbs. The total polyphenols could represent a specific parameter for the nutritional values of plants. Further investigations are proposed to evaluate the relative composition of individual polyphenolic compounds from oregano, sage and basil.

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