

## ANTIOXIDANT ACTIVITY AND SECONDARY METABOLITES IN DIFFERENT EXTRACTS OF *EUPHRASIA OFFICINALIS* L. GROWING IN BULGARIA

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### ABSTRACT

*Euphrasia officinalis* L. (eyebright) belongs to the family *Scrophulariaceae*, distributed in Europe, Northern and Western Asia and North America. It possesses a wide spectrum of therapeutic activities - anti-inflammatory, antibiotic, antioxidant and astringent, which is related to the variety of biologically active substances in that plant: iridoid glycosides, flavonoids, essential oils, phenolic acids, several B vitamins, vitamins A, C, D and E, and tannins.

The aim of the present study was to evaluate the total phenol and flavonoid contents and antioxidant activity measured by phosphomolybdenum method of chlorophorm and methanol extracts obtained by Soxhlet extraction from *Euphrasia officinalis*. The highest phenolic content and total antioxidant activity were established in methanol extract (105.23±6.05 mg/g gallic acid equivalent and 239.08±3.92 mM  $\alpha$ -tocopherol/g extract, respectively). The amount of flavonoids in the methanol extract was two-fold lower than in the chlorophorm extract. To the best of our knowledge, this is the first report that indicates total phenolic and flavonoid contents and total antioxidant capacity of extracts from *Euphrasia officinalis* plants.

**Key words:** *Euphrasia officinalis* L., Soxhlet extraction, secondary metabolites, antioxidant activity

### INTRODUCTION

Plants have been used traditionally in folk medicine for the treatment and prophylaxis of different disorders (Prior, 2003). Polyphenols are commonly found in both edible and non-edible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kahkonen et al., 1999). The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Morel et al., 1994; Rice-Evans et al., 1997). The modern medicine often utilizes phytochemicals with good antioxidant properties for the prevention and treatment of diseases. Folk medicinal remedies in Bulgaria go together with the traditional medicine and medicinal plants are widely used in the everyday diet of the population in the form of teas, infusions and extracts (Kiselova et al., 2006).

*Euphrasia officinalis* L., commonly known as eyebright, belongs to the family *Scrophulariaceae*, distributed in Europe, Northern and Western Asia and North America. It grows well on moist and chalky soils, on Alpine and sub-alpine meadows with frequent snow periods. *Euphrasia officinalis* is used in the treatment of eye infections, disorders of the stomach, jaundice, diabetes, headache and respiratory diseases. It possesses a wide spectrum of therapeutic activities - anti-inflammatory, antibiotic, antioxidant and astringent, which is related to the variety of biologically active substances in that plant: iridoid glycosides, flavonoids, essential oils, phenolic acids, several B vitamins, vitamins A, C, D and E, and tannins (Gorji, 2003; Blazics and Kéry, 2007).

The aim of this study was to evaluate the total phenol and flavonoid contents and antioxidant activity of chlorophorm and methanol extracts from plants of *Euphrasia officinalis*.

## MATERIAL AND METHODS

### Plant material

Above-ground parts were collected from mature plants of *Euphrasia officinalis* L. harvested in the foot of mountain Stara planina, near the town of Lovech, Bulgaria. The plant material was dried in the shade and ground in a grinder.

### Soxhlet extraction

Three grams of powdered aerial parts of *E. officinalis* L. were extracted by Soxhlet extraction with 30 ml chloroform for 8 h until full colourlessness and then the same plant material was used for second extraction with methanol. Solvents were removed by rotary evaporation and dryness. Extracts were concentrated, dried and kept in the dark at 4° C for further experiments.

### Determination of total phenolic content

Total phenolic content in the crude extracts of *E. officinalis* L. was determined according to Singleton et al., 1999 with modifications. Aliquots of 0.1 ml of chloroform and methanol extracts with concentration 1 mg.ml<sup>-1</sup>, were mixed with 1.5 ml Folin–Ciocalteu reagent (1:10 diluted in dH<sub>2</sub>O) and 1.4 ml sodium carbonate 7.5 %. The samples were incubated at room temperature for 30 min and after the reaction the absorbance was read at 765 nm by using Shimadzu UV 1800 spectrophotometer. Total phenolic content was expressed as mg.g<sup>-1</sup> gallic acid equivalents (GAE) of extract.

### Determination of total flavonoid content

Total flavonoid content was performed according to Chang et al., 2002 with slight modifications. Aliquots of 0.5 ml of chloroform and methanol extracts with concentration 1 mg.ml<sup>-1</sup>, were mixed with 1.4 ml methanol, 0.1 ml aluminum chloride 10 %, 0.1 ml potassium chloride 1 M and 2.8 ml distilled water. The mixture was incubated at room temperature for 30 min and after the reaction the absorbance was measured with a Shimadzu UV 1800 spectrophotometer at 415 nm. Total flavonoid content was expressed as mg.g<sup>-1</sup> quercetin equivalents of extract.

### Determination of total antioxidant activity

The total antioxidant capacity of the extracts was evaluated by the method of Prieto, Pineda, and Aguilar, 1999 with slight modifications. The antioxidant capacity of the extracts was measured spectrophotometrically using a phosphomolybdenum method, based on the reduction of Mo(VI) to Mo(V) by the sample analyzed and the subsequent formation of green phosphate/Mo(V) compounds with a maximum absorption at  $\lambda = 695$  nm. A 0.25 ml aliquot of sample solution (1 mg.ml<sup>-1</sup>) was mixed with 2.5 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm, against a blank solution by using a Shimadzu UV 1800 spectrophotometer. A blank solution contained 2.5 ml of reagent solution and the appropriate volume of methanol used for the dissolution of the samples and it was incubated under the same conditions as the other samples. The total antioxidant capacity was expressed as equivalents of  $\alpha$ -tocopherol acetate mM.g<sup>-1</sup> of extract.

### Statistical analysis

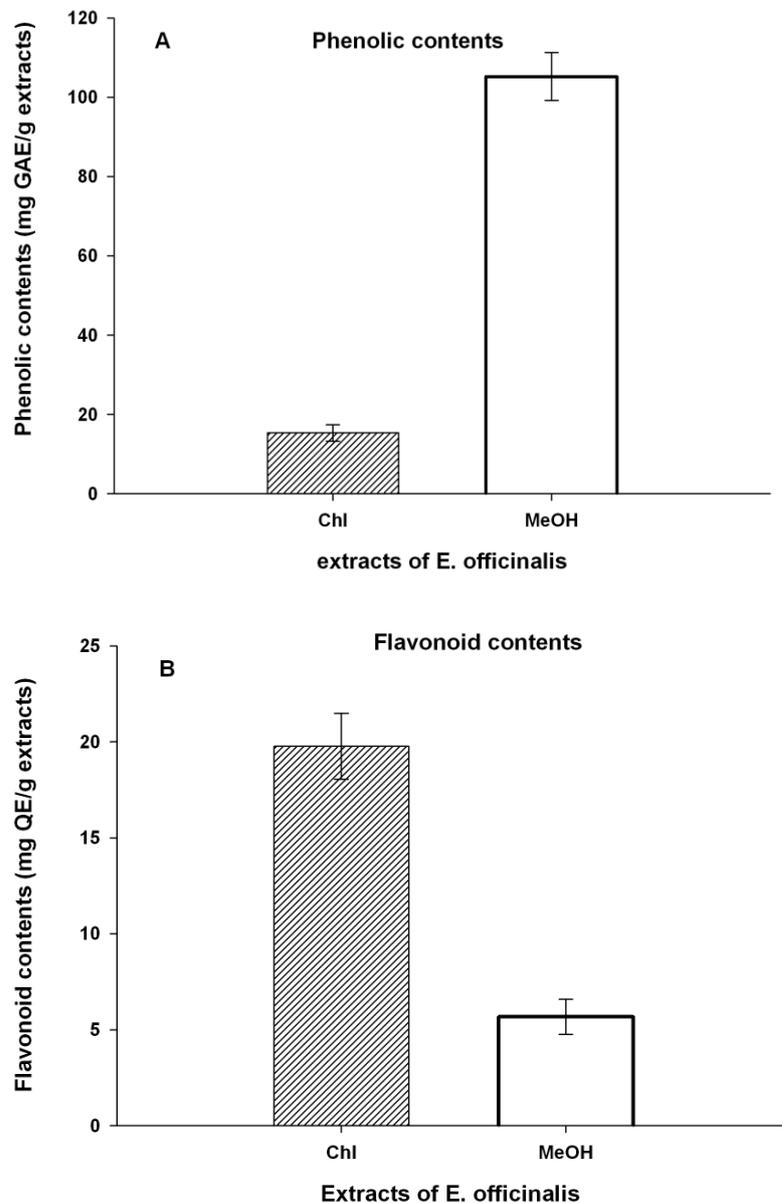
Presented data for all experiments are average values from at least four independent experiments and are compared by standard error of the means (S.E.M.).

## RESULTS AND DISCUSSION

Chloroform and methanol extracts of *E. officinalis* L. grown in Bulgaria obtained by Soxhlet extraction were studied for their total phenolic and flavonoid content and total antioxidant activity. Antioxidant activity of phenols and flavonoids depends on their structure, substitution pattern of hydroxyl groups, polarity of solvents and methods used for extraction. The highest phenolic contents and total antioxidant activity measured by phosphomolybdenum method were measured in methanolic extract of *E. officinalis* (105.23 ± 6.02 mg GAE/g extract and 293.08 ± 3.92 mM  $\alpha$ -

tocopherol/g extract, respectively), compared to chlorophorm extract, which content was approximately four times less than the total phenols and antioxidant activity (Figure 1, A and C). Water extract received by aerial part of *E. officinalis* L. exhibited an intermediate antioxidant activity measured by ABTS<sup>+</sup> and total phenol contents, compared to another 23 Bulgarian medicinal plants and four foreign species (Kiselova et al., 2006).

Recently, in purified extract of *E. officinalis* were identified different flavonoids such as miricetin, quercetin, luteolin, apigenin, kaempferol and acacetin measured by HPLC (Vukics et al. 2006). According to Vukics et al. 2006, using methanol as solvent seemed to have higher selectivity for flavonoid aglycones compared to other solvents such as ethyl acetate and acetone. On the contrary, in our study the amount of flavonoids in the methanol extract was two-fold lower than in the chlorophorm extract (Figure 1, B). On the other hand methanolic extract of *E. officinalis* L. received by Soxhlet extraction showed high phenolic content and total antioxidant activity, which may be due to the presence of different secondary metabolites (Figure 1, A and C).



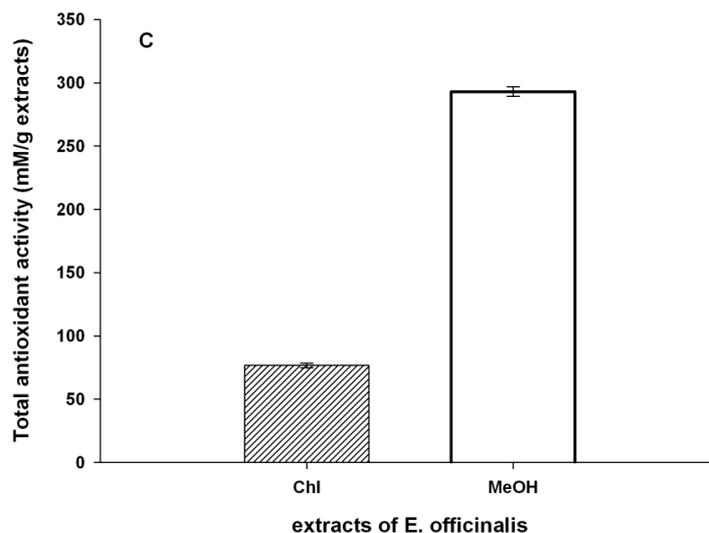


Figure 1. Concentrations of secondary metabolites and antioxidant activity in chlorophorm and methanol extracts of *Euphrasia officinalis* L. obtain by Soxhlet extraction. (A) Total phenolic contents; (B) Total flavonoid contents; (C) Total antioxidant activity.

In purified methanol extract of *E. officinalis* was identified caffeoyl-derivatives, iridoids and phenolics/flavonoids by means of HPLC and LC-MS/MS methods, which provided the highest scavenging activity in both DPPH and ABTS assays. The scavenging effect of *Euphrasia officinalis* L. was a combination of the iridoids and phenolics/flavonoids, though the phenolic compounds basically determined this effect (Blazics and Kéry, 2007). According to Grzegorzczuk et.al 2007 differences in antioxidant properties of plants in different biological systems may be attributed to the presence of different secondary metabolites and methods used for extraction.

## CONCLUSION

Based on the results described above, it is concluded, that methanolic extract of *Euphrasia officinalis* L. showed greatest antioxidant activity, which correlated with their high phenolic content. Further investigations are needed for identification of biological active compounds and modulation of their synthesis, in order to obtain higher yield of secondary metabolites.

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