

Screening of antioxidant activity of the eyebright herb, *Euphrasia officinalis**

Nada Kovačević, Milica Kalezić, Mirjana Simić

Department of Pharmacognosy, Faculty of Pharmacy,
University of Belgrade, Vojvode Stepe 450, Belgrade.

Summary

The aim of this work was investigation of antioxidant activity of the eyebright herb. It is known that this plant (*Euphrasia officinalis* L., Scrophulariaceae) contains a mixture of polyphenolic components. Usually antioxidant activity is connected with the presence of this kind of plant constituents. For investigation, dry methanol extract of previously defeated herb was prepared. The antioxidant activity was investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. Before, the determination of polyphenolic constituents in the plant material as well as, in the extract was done. The herb contains 1.27% of total polyphenols, while the concentration of these substances in methanol extract was 14.54%. Besides this, content of tannins (0.97% in the herb; 10.42% in the extract), total flavonoids (0.59% in the herb; 1.67% in the extract) and anthocyanins (0.75 mg/g of the herb; 1.45 mg/g of the extract) was determinate. The methanol extract showed concentration dependent, significant DPPH radical scavenging activity. IC₅₀ value for the dry methanol extract of the eyebright herb was 11.2 µg/ml.

Keywords: *Euphrasia officinalis*, herb, methanolic extract,
polyphenolic constituents, DPPH radical scavenging activity.

* Rad je saopšten na Kongresu farmaceuta Bugarske; 3-5. jun 2005. Sofija, Bugarska
This paper has been presented at Congress of Pharmacist of Bulgaria, 3-5. June, Sofia,
Bulgaria

Introduction

Eyebright (*Euphrasia officinalis* L., Scrophulariaceae) is annual hemiparasitic herb, which grows in a large part of Europe (1). This plant is well known in traditional medicine. Mainly, eyebright is used externally, as lotion or eye-baths. It is used for prevention and treatment of different disorders connected with inflammation of blood vessels, eyelids and conjunctiva (2). Up to now, there are no scientific and clinical claims for its efficacy, but there are some data about chemical constituents of *E. officinalis*. It is confirmed that herb contains iridoide monoterpenes (aucubin, catalpon, euphosid), lignans (dehydrodiconiferyl-4- β -D-glucoside), flavonoids (7-O-galactosides and 7-O-rhamnogalactosides of apigenin, luteolin and chrysoeriol), tannins and essential oil (3).

In this study the antioxidant activity of *E. officinalis* herb, using DPPH free radical assay was investigated.

Materials and Methods

Aerial parts were collected in the flowering stage at the Tara Mountain at the end of September 2004. After extraction with petroleum ether, plant material was extracted with methanol (70%; v/v), by maceration at room temperature during 24 h, filtrated and solvents evaporated under reduced pressure up to dryness. Yield of petroleum extract and methanol extract were 3.3% and 28.3%, respectively. Chemical composition of extracts was monitored by TLC.

The contents of total polyphenols and tannins in dry plant material and methanol extract were determined by Folin-Ciocalteu method and calculated as catechin (4). The content of flavonoid heterosides was evaluated spectrophotometrically, according to DAB 10 monograph *Crataegi folium et flores* and expressed as hyperoside (5). The content of anthocyanidins was determined spectrophotometrically. After hydrolysis, by heating with 2M HCl during 60 minutes, anthocyanidins were extracted with butanol and the absorption was measured at 550 nm. Results are expressed as cyanidine (6).

DPPH radical scavenging activity was measured according to the method described by Cuendet et al. (7), based on the transformation of stable DPPH \cdot to its reduced form (DPPH-H) in the reactions with an antioxidant compound, which can donate hydrogen. The changes in colour (from deep-violet to light-yellow) were measured spectrophotometrically.

Dry methanol extract was re-dissolved in methanol to obtain concentrations of 2.5-60 μ g/ml. Sample solutions (4 ml) containing the

methanol extract at different concentrations were mixed with 1 ml of 0.5 mmol/l DPPH radical (Sigma) solution in methanol. The mixture was left to react in the dark and at room temperature, for 30 min. Blank sample contained the same amount of methanol and DPPH as the test solutions. The absorbance was measured at 517 nm after 30 min and the activity was calculated. Dose-response curves were constructed and IC₅₀ values were calculated. All measurements were performed in triplicate. Ascorbic acid (La Chema) and rutin (Fluka) were used as reference compounds.

Results

According to obtained results, dry eyebright herb contained 1.27% of total polyphenols. Different classes of polyphenolic constituents were determinate separately; the investigated sample of the *E. officinalis* herb contained 0.97% of tannins, 0.59% of total flavonoids and 0.75 mg/g anthocyanins. Similar analyses were done in the extract. Methanol extract of the herb contained 14.54% of total polyphenols. Separately, there were 10.42% of tannins, 1.67% of total flavonoids and 1.45 mg/g anthocyanins.

Table I Content of polyphenolic constituents in herb and methanol extract of *Euphrasia officinalis*

Tabela I Količina polifenolnih sastojaka u herbi i u metanolnom ekstraktu vidovčice, *Euphrasia officinalis*

	Total polyphenols	Tannins	Total flavonoids	Anthocyanins
Dry herb	1.27±0.02%	0.97±0.001%	0.59±0.3%	0.75±0.4 mg/g
Methanol extract	14.54±0.003%	10.42±0.01%	1.67±0.6%	1.45±0.7 mg/g

Table II DPPH free radical scavenging activity of methanol extract of *Euphrasia officinalis* herb

Tabela II Intenzitet neutralizacije DPPH radikala primenom metanolnog ekstrakta herbe vidovčice

Concentration of extract	2.5 µg/ml	5 µg/ml	10 µg/ml	20 µg/ml	40 µg/ml	60 µg/ml
DPPH radical scavenging activity	9.1±2.0%	19.9±1.1%	37.3±1.9%	77.1±0.4%	92.0±0.9%	91.7±0.6%

The methanol extract of eyebright herb showed a strong radical scavenger activity. Concentration dependent DPPH radical scavenging activity was obtained. The highest DPPH free radical scavenging activity (92%) was observed with a concentration 40 µg/ml, when a reaction reached a plateau. This percentage can be considered as a full scavenger activity, because after completing the reaction, the final solution always possesses some yellowish colour and therefore its absorption inhibition, compared to colourless methanol solution, can't reach 100% (8). IC₅₀ value for investigated extract was 11.2 µg/ml. The antioxidant activity of *E. officinalis* extract was lower than those obtained for referent substances: ascorbic acid (IC₅₀ was 4.1 µg/ml) and rutin (IC₅₀ was 2.5 µg/ml). Radical scavenger activity of *E. officinalis* herb extract, probably, has to be connected with the content of polyphenolic constituents of eyebright herb.

Acknowledgements. This work is part of the Project No. 1568, which is supported by the Ministry of Sciences of Serbia.

References

1. Tutin T.G. et al. Flora Europaea. Volume 3. London, New York, Cambridge: University Press, 1972.
2. Wichtl M. Teedrogen und Phytopharmaka. Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage. 4. Auflage, Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH, 2002.
3. Duke J.A. Handbook of medicinal herbs. Boca Raton: CRS Press, 1985: 193.
4. Makkar H.P.S., Hagerman, A., Harvey-Mueller, I. Quantification of Tannins in Tree Foliage – A Laboratory Manual. Vienna: FAO/IAEA, 2000.
5. Deutsches Arzneibuch. 10. Ausgabe, Deutscher Apotheker Verlag Stuttgart, Govi-Verlag GmbH Frankfurt, 1991.
6. Harborne J.B. Phytochemical Methods, London: Chapman and Hall, 1973.
7. Cuendet M., Hostettmann, K., Potterat, O. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helv Chim Acta 1997; 80: 1144-52.
8. Miliuskas G., Venskutonis P.R., van Beek T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004; 85: 231-7.

Preliminarno ispitivanje antioksidantne aktivnost herbe vidovčice, *Euphrasia officinalis*

Nada Kovačević, Milica Kalezić, Mirjana Simić

Institut za farmakognoziju, Farmaceutski fakultet,
Univerzitet u Beogradu, Vojvode Stepe 450, Beograd

Kratak sadržaj

Vidovčica (*Euphrasia officinalis* L., Scrophulariaceae) je poluparazitska biljna vrsta, rasprostranjena u Evropi. Tradicionalno se primenjivala eksterno, naročito kod inflamacija oka, u vidu kupki ili losiona.

Pošto se antioksidantna aktivnost biljnih ekstrakata povezuje sa sadržajem polifenolnih sastojaka, određen je njihov sadržaj u ispitivanoj herbi i ekstraktu. Utvrđeno je da ispitivani uzorak herbe vidovčice sadrži 1,27% ukupnih polifenola. Posebno je određen sadržaj tanina (0,97%), ukupnih flavonoida (0,59%) i antocijana (0,75 mg/g).

Antioksidantna aktivnost herbe vidovčice je ispitivana na osnovu neutralizacije slobodnih radikala primenom DPPH (2,2-difenil-1-pikrilhidrazil) testa. Posle obezmašćivanja herbe, pripremljen je metanolni ekstrakt (70%; v/v) i uparen do suva. Metanolni ekstrakt je sadržao 14,54% ukupnih polifenola i to: 10,42% tanina, 1,67% ukupnih flavonoida i 1,45 mg/g antocijana. Utvrđeno je da metanolni ekstrakt herbe vidovčice poseduje izraženu sposobnost uklanjanja slobodnih radikala. Ovaj ekstrakt je ispoljio dozno zavisnu sposobnost neutralizacije DPPH radikala. IC₅₀ vrednost je iznosila 11,2 µg/ml. Može se pretpostaviti da su za ovakvu aktivnost ispitivanog ekstrakta odgovorni polifenolni sastojci vidovčice.

Ključne reči: *Euphrasia officinalis*, herba, metanolni ekstrakt,
polifenolni sastojci, neutralizacija DPPH radikala.
