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FREE RADICAL SCAVENGING AND MEMBRANE PROTECTIVE EFFECTS OF METHANOL EXTRACTED FRACTIONS OF PARSLEY

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Free radical scavenging and membrane protective activity of the methanol extracts of the herb of *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill., parsley were evaluated with chemiluminometric and ascorbic acid induced lipidperoxidation methods. Apiin was used as reference material. Good correlation was observed between the chemiluminometric and the membrane protective activity of the samples.

Keywords: *Petroselinum crispum*, parsley, antioxidant, free radical scavenging, lipidperoxidation

Parsley [*Petroselinum crispum* (Mill.) Nym. ex A. W. Hill.] is a small bienial flowering plant bearing greatly divided pinnately compound leaves. Parsley is extensively employed as a culinary herb for garnishing and seasoning. The seeds have strong diuretic activity due to the high essential oil content (WARNCKE, 1992). The leaves are wildely used as spice. Characteristic constituents are essential oils (apiol, myristicin), flavonoids (apiin, luteolin-, apigenin-glycosides), cumarines (bergapten, imperatorin) and ascorbic acid (HÄNSEL et al., 1994).

Considering the present information about spices – paying more attention to their free radical scavenger and antioxidant activity – the reconsideration of these plants is a very promising area in understanding their value. Since in the literature no information could be found about the antioxidant activity of parsley, we intended to provide evidence that *Petroselinum crispum* (Mill.) Nym. ex. A. W. Hill. extracts have free radical scavenging and membrane protective effects.

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1. Materials and methods

1.1. Plant material

Petroselinum crispum (Mill.) Nym. ex A. W. Hill. samples were purchased from a well-known vegetable market in Budapest and were identified at the Semmelweis University of Medicine, Institute of Pharmacognosy.

1.2. Reagents

Luminol (5-amino-2,3-dihydro-1,4-phtalazinedione No.: A4685) and micropreroxidase (No.: M9635) were purchased from Sigma-Aldrich Kft., while apiin (No.: 25635) from Carl Roth Gmbh. The unlabelled chemicals and reagents were of analytical grade from Reanal Finomvegyszergyár Rt. Budapest.

1.3. Extraction and purification

The aerial part of parsley (200 g) was extracted by the Soxhlet technique with hexane, chloroform, ethylacetate and methanol. Concentration of the methanol extract under pressure resulted in 21.3 g of dry material. Stock solutions (0.5 g l^{-1}) were prepared with methanol and water and were used in the chemiluminometric and anti-lipoperoxidant experiments. An aliquot of 6.0 g, part of the dried methanol extract, was separated on Sephadex LH 20 column (length: 75 cm; I.D: 3.5 cm; flow rate: 4 ml min^{-1}) using methanol as solvent. Six fractions were obtained (300 ml each): S1 (0.7 g), S2 (1.2 g), S3 (0.8 g), S 4 (1.1 g), S5 (0.9 g) and S6 (0.5 g). The solvent was evaporated under pressure. Methanol and water stock solutions were prepared (0.1 g ml^{-1}) from these fractions. Total flavonoid content was measured according to DAB 10 (1991) and was expressed as hyperoside mg l^{-1}. Methanol solutions were used in the chemiluminometric, while water solutions in the anti-lipoperoxidant experiments (FEJES et al., 1999).

1.4. Free radical scavenging activity

Non-specific free radical scavenging activity was measured in Lumat LB9501 luminometer using a chemiluminescence method (BLÁZOVICS & FEHÉR, 1995). Experimentation time was 30 s, and the procedure was carried out at room temperature. H_2O_2 concentration was 5×10^{-5} mol l^{-1}, luminol concentration was 0.07 mmol l^{-1}, Na_2CO_3 concentration was 1.18 mmol l^{-1}, the microperoxidase concentration was 3×10^{-7} mol l^{-1}. The emitted light signals were counted over the preselected time periods (30 s) and were then integrated (chemiluminescence intensity). The changes of chemiluminescence intensity of the H_2O_2/•OH-luminol system at different sample concentrations were measured. The background chemiluminescence was evaluated with

addition of equal quantity of methanol to the samples. The percentage of the free radical scavenging activity was calculated as follows:

Free radical scavenging activity = 1-(chemiluminesescence intensity of samples/background chemiluminescence intensity).

1.5. Anti-lipoperoxidant activity

Homogenates of the brains of young male Wistar albino rats weighing 150–200 g were prepared by the method of FEHÉR and co-workers (1985). Protein concentration of the brain homogenates was assayed by the methods of LOWRY and co-workers (1951).

Non-enzymatic lipid peroxidation was studied according to BLÁZOVICS & FEHÉR (1992). The total volume was 0.5 ml and contained protein suspension (1 mg ml^{-1}), 500 mmol l^{-1} tris-maleate buffer (pH 6.8), 50 mmol l^{-1} KH_2PO_4, 5×10^{-5} mmol l^{-1} of ascorbic acid, plus various concentrations of parsley fractions diluted in water. Temperature was 37 °C, the incubation time was 20 min. Malondialdehyde production was monitored by the thiobarbituric acid test of OTTOLENGHI (1959). A molar absorption coefficient E_{532} 1 cm of 156 mmol l^{-1} cm^{-1} was used.

1.6. Statistical analysis

The in vitro experimental results were expressed as the mean ±S. D. of three parallel measurements (P>95%).

2. Results

Figure 1 shows the results obtained with methanol and water solutions of the dried methanol extract of the herb tested with chemiluminometric and lipidperoxidation methods. From the first figure an obvious tendency can be deduced. The higher the flavonoid concentration is the greater the increase in the free radical scavenging and membrane protective activity. However, there are differences between the samples. The herb sample shows the highest activity rates. It scavenges 21±8% at the lowest concentration and then shows a rapid increase to 79±8% at the next concentration (0.5 mg l^{-1}). At the last concentration (2 mg l^{-1}) this sample scavenges all of the radicals present.

Apiin standard also shows a slight activity by 0.5 mg l^{-1} concentration 42±7% and it reaches 88±8% at the highest concentration (2 mg l^{-1}).

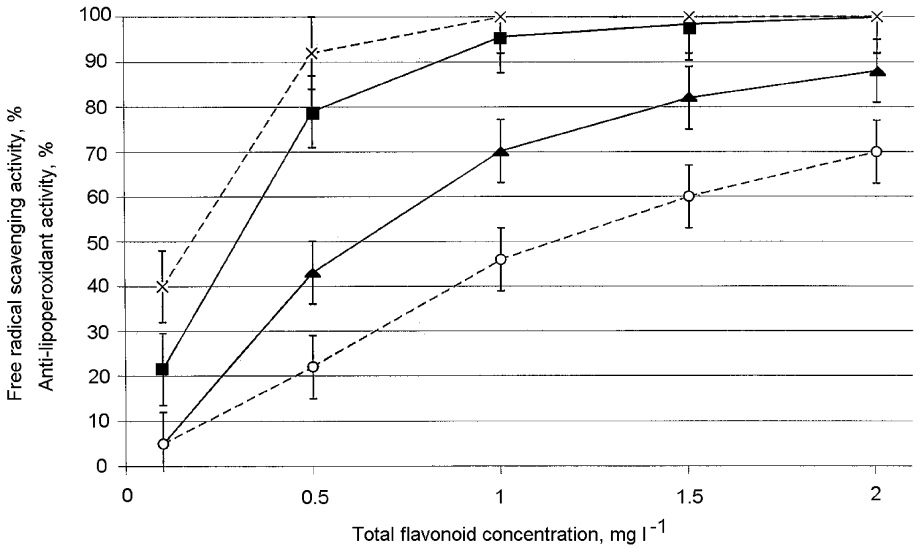

Fig. 1. Comparison of the free radical scavenging and anti-lipoperoxidant effects of the herb extract of *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. and apiin. Results are mean ±S.D. of three parallel measurements. —▲—: free radical scavenging activity of apiin; —■—: free radical scavenging activity of the herb; —❍—: anti-lipoperoxidant activity of apiin; —×—: anti-lipoperoxidant activity of the herb

Table 1

Mathematical analysis of the curves in Fig. 1

Curves in Fig. 1	Linear correlation	r^2
Free radical scavenging activity of apiin	y=1.0246x+1.7718	0.9844
Free radical scavenging activity of the herb	y=0.5366x+1.9115	0.9517
Anti-lipoperoxidant activity of apiin	y=0.9038x+1.6144	0.9952
Anti-lipoperoxidant activity of the herb	y=0.3107x+1.9611	0.8716

$y = \log_{10}$ (free redical scavenging or anti-lipoperoxidant activity), $x=\log_{10}$ (concentration),
r^2 = correlation coefficient

In comparison to the activity of these samples, the herb sample has better activity in the biological system than in the chemical one. At a concentration of 1 mg l^{-1} total anti-lipoperoxidant activity was measured. Meanwhile apiin was less effective in the biological system than in the chemical one. Our reference material apiin had 70±8%

anti-lipoperoxidant activity at the highest concentration. Mathematical analysis of the results in Fig. 1 according to log-log transformation between sample concentration and chemiluminescence intensity or anti-lipoperoxidant activity showed a linear correlation (Table 1). Interestingly, the correlation coefficient was always higher, when apiin standard was tested. It underlines that the herb sample act in a more complex way than apiin does.

In Fig. 2, the free radical scavenging and the anti-lipoperoxidant activity of the purified methanol herb extracts (S1–S6) are compared. Apiin was used as reference material. The figure shows that the anti-lipoperoxidant activity was always higher than the free radical scavenging activity, apart from fraction S4 and apiin. Apiin showed a 57±4% quenching and a 45±5% anti-lipoperoxidant effect. S4 scavenged all the free radicals, which were present in the chemiluminometric system and reduced the malondialdehyde production by 85±4%. S2 and S3 were mainly effective in the lipidperoxidation experiments (S2: 51±4%, S3: 52±4%). The six fractions contain different amount of flavonoids, which can be seen in Table 2.

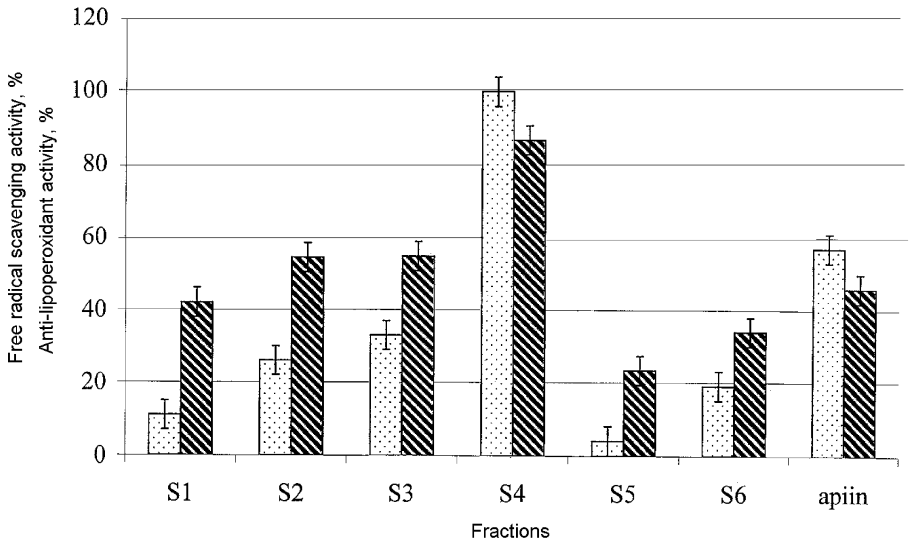

Fig. 2. Free radical scavenging and anti-lipoperoxidant effects of the herb extracts of *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. purified by Sephadex LH20 column chromatogtraphy, compared to apiin. Results are mean ±S.D. of three parallel measurements. ⬚: free radical scavenging activity; ▨: anti-lipoperoxidant activity

Table 2

Total flavonoid content of Petroselinum crispum *(Mill.) Nym. ex A. W. Hill purified fractions*

	Samples						
	S1	S2	S3	S4	S5	S6	Apiin
Flavonoid content in hyperoside (mg l^{-1})	0.03±0.01	0.24±0.01	0.28±0.03	2.76±0.01	0.05±0.02	0.19±0.03	1±0.02

Results are mean ±S.D. of three parallel measurements

Higher flavonoid concentration resulted in better free radical scavenger and anti-lipoperoxidant activities in both experiments. The correlation coefficient between the total flavonoid content and the free radical scavenging capacity was r=0.959141. Weaker correlation was discovered (r=0.834051) between the total flavonoid content and the anti-lipoperoxidant activity. S4 contained 2.76±0.01 mg l^{-1} of flavonoids, which scavenged 100% of the radicals present, while S5 contained 0.05±0.03 mg l^{-1} of flavonoids and scavenged 5% of the radicals.

3. Conclusion

Since the phytotherapeutical effects of parsley have not yet been fully confirmed and analysed (HÄNSEL et al., 1994), this investigation suggests one possible way for reconsideration of the pharmaceutical activities. Free radical scavenging and membrane protective effects of *Petroselinum crispum* (Mill.) Nym ex A. W. Hill. herb extracts were investigated. Apiin, which is the main flavonoid constituent of the herb (FEJES et al., 1998), was used as reference material. Analysis of the experimental results and the presumable compounds of the investigated crude plant extracts lead to the following conclusions.

Petroselinum crispum (Mill.) Nym. ex A. W. Hill. and apiin had free radical scavenging activity in the chemiluminometric tests and anti-lipoperoxidant effect on ascorbic acid induced lipid peroxidation in vitro. Comparing the chemiluminometric results with the lipidperoxidation studies, the following results should be mentioned.

Apiin demonstrated higher free radical scavenging potential in the chemiluminometric studies than membrane protective activity under the anti-lipoperoxidant conditions. Parsley extracts, however, had better protective effects in the anti-lipoperoxidant measurements, than scavenging activity in the chemiluminometric studies.

The plant sample was always more effective in both experiments when its total flavonoid concentration was the same as that of the reference material.

Both conclusions can be explained by the fact that in a plant extract there are several similar types of molecules, which can react synergically, or enrich one another. This is the reason why apiin and the almost pure apiin containing S4 sample showed lower activity in the biological environment. Probably the present compounds (e.g.: polyphenols) in the parsley samples react in a more complex way and have more target points in the biological system than they have in the chemical one. They not only scavenge the radicals in the biological medium, but they can brake the free radical chain reactions, they could chelate transitional metal ions and quench singlet oxygen. Further studies are in progress to analyse the synergetic effects of these compounds.

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References

BLÁZOVICS, A. & FEHÉR, E. (1992): Role of free radical reactions in experimental hyperlipidemia in the pathomechanism of fatty liver. -in: CSOMÓS, G. & FEHÉR, J. (Eds) *Free radicals and the liver*. Springer-Verlag, Berlin, Heidelberg, pp. 96–123.

BLÁZOVICS, A. & FEHÉR, J. (1995): Természetes antioxidánsok és szöveti regeneráció (Gyógyhatás és reakciómechanizmus) II. rész. (Natural antixodants and tissue regeneration (Curative effects and reaction mechanisms.) Part II.) *Fitoterápia, 1*, 171–176.

DAB 10 (1991): *Deutsches Arzneibuch*, 10. Ausgabe. Deutscher Apotheker Verlag, Stuttgart. Govi-Verlag Gmbh, Frankfurt/M.

FEHÉR, J., BLÁZOVICS, A., CORNIDES, A. & VERECKEI, A. (1985): Effect of (+)-cyanidanol-3 on rat brain lipid peroxidation. *Br. J. Exp. Path*., *66*, 161–164.

FEJES, SZ., BLÁZOVICS, A., LEMBERKOVICS, É., PETRI, G., SZŐKE, É. & KÉRY, Á. (1999): Free radical scavenging and membrane protective effects of methanol extracts from *Anthriscus cerefolium* L. (Hoffm.) and *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. *Phytotherapy Res.*, in press.

FEJES, SZ., KÉRY, Á., BLÁZOVICS, A., LUGASI, A., LEMBERKOVICS, É., PETRI, G. & SZŐKE, É. (1998): A *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. in vitro antioxidáns hatásának vizsgálata. (Investigation of the in vitro antioxidant effect of *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill.) *Acta pharm. Hung.*, *68*, 150–156.

HÄNSEL, R., KELLER, K., RIMPLER, H. & SCHNEIDER, G. (1994): Petroselinum. -in: *Hagers Handbuch der pharmazeutischen Praxis.* 5. Auflage, Springer-Verlag, Berlin, Heidelberg, Vol. 6, pp. 105–119.

LOWRY, A. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951): Protein measurement with the folin-phenol reagents. *J. biol. Chem.*, *193*, 265–275.

OTTOLENGHI, A. (1959): Interaction of ascorbic acid on mitochondrial lipides. *Arch. Biochem. Biophys.*, *79*, 355–363.

WARNCKE, D. (1992): *Untersuchungen über die Zusammensetzung der aetherisehen Öle von* Petroselinum crispum *(Mill.) A. W. Hill. und* Petroselinum segetum *(L.) Koch unter besonderer Berücksichtigung von Handelsdrogen und Handelsölen*. Dissertation, Würzburg, Fachbereich Biologie.