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# Free Radical Scavenging and Membrane Protective Effects of Methanol Extracts from *Anthriscus cerefolium* L. (Hoffm.) and *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill.

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**The antilipoperoxidant activity of *Anthriscus cerefolium* L. (Hoffm.), chervil, *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill., parsley extracts were evaluated with ascorbic acid induced lipid peroxidation on rat brain homogenates. These results are completed by the antiradical potential of these extracts against a solution of $OH^{\cdot}$ radical. In all cases luteolin-7-O-glucoside was used as a reference material.** 

*Keywords: Anthriscus cerefolium*; chervil; *Petroselinum crispum*; parsley; antioxidant; free radical scavenging; lipidperoxidation.

## INTRODUCTION

Deterioration of food quality via oxidative rancidity is often the primary factor limiting the shelf life of food products, even in foods that have low fat content. For many food products, the membrane constituents of the cells comprising the food are the primary sites of peroxidative damage. This is probably related to the surface exposure of membrane phospholipid molecules to the aqueous phase containing peroxidation-initiating species (Hiramatsu *et al.*, 1997). The extent to which oxidation of fatty acids and their esters occurs in foods depends on the chemical structure of the fatty acid, the nature of food processing, the temperature at which the foods are stored and/or cooked, and the minor constituent antioxidants (Auroma and Cuppet, 1997). Incorporation of antioxidants into food products may impart significant health benefits to consumers in addition to stabilizing the food product. The need to screen large numbers of compounds for antioxidant activity requires rapid and sensitive assays for evaluating the free radical scavenging, membrane protective activities. This report described *in vitro* screening experiments of two Apiaceae medicinal plants to address this need.

Parsley (*Petroselinum crispum* (Mill.) Nym. ex A. W. Hill.) has been used as a popular spice and vegetable. The seeds have a strong diuretic activity due to its high essential oil content. The leaves are widely used as a spice. The characteristic constituents are essential oil (apiol, miriszticin), flavonoids (apiin, luteolin-, apigenin-glycosides) and cumarines (bergapten, imperatorin) (Hänsel *et al.*, 1994).

Chervil, *Anthriscus cerefolium* L. (Hoffm.) belonging to the Apiacae family has been used formally as a drug (Herba cerefolii), but at present its principal use is as a flavouring agent for culinary purposes. In folk medicine, however, its herb was used to alleviate circulation disorders (Bremness, 1989). Characteristic constituents of the herb are flavonoids (apiin, luteolin-glycosides) (Tozaburo and Masao, 1979), and essential oil (methylchavicol = estragole, 1-allyl-2,4-dimethoxybenzene) (Zwaving *et al.*, 1970; Simándi *et al.*, 1996). Fractional distillation was used in a Soxhlet apparatus to yield a flavonoid-rich methanol extract from each herb.

A well-known test method, chemiluminescence, was used to demonstrate the non-specific free radical scavenging activity. The membrane protective activity was measured with ascorbic acid induced lipid peroxidation on rat brain microsomal fraction. Luteolin-7-O-glucoside was used as a reference material.

## MATERIALS AND METHODS

**Plant material.** *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. was purchased from a well-known vegetable market in Budapest.

*Anthriscus cerefolium* L. Hoffm. samples were collected before the full flowering state from the hills surrounding Budapest, Hungary. Before they were dried in shade, the roots were removed from the herb. The dried herbs were ground before extraction.

**Reagents.** Luminol, microperoxidase and luteolin-7-O-

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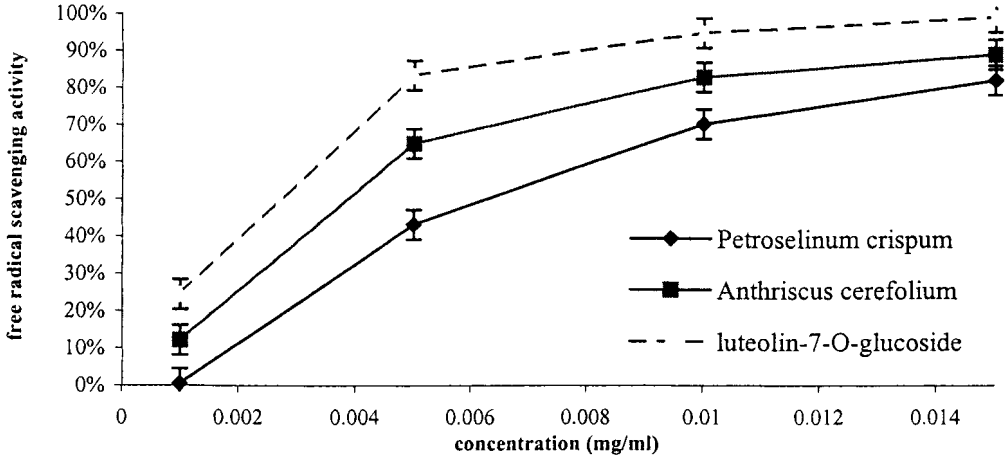


**Figure 1.** Free radical scavenging effect of the methanol extracts from *Anthriscus cerefolium* L. Hoffm. and *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. in $H_2O_2$/·OH-luminol system. Results are mean ± SD of five parallel measurements, $p < 0.05$ compared with control.

glucoside were purchased from Sigma Chemical. The unlabelled chemicals and reagents were analytically pure from Reanal Finomvegyszergyár Rt. Budapest.

**Extraction and purification.** 100 g of the herb parts was extracted by the Soxhlet technique successively with hexane, chloroform, ethylacetate and methanol. Concentration of the methanol extracts under pressure gave 19.5 g from chervil and 22.4g from parsley, respectively. Methanol and water stock solutions were prepared (0.2g/mL). Methanol solutions were used in the chemiluminometric, and water solutions in the antilipoperoxidant experiments.

**Free radical scavenging activity.** Non-specific free radical scavenging activity was measured in a Lumat LB9501 luminometer using a chemiluminescence method (Blázovics and Fehér, 1995). The experimentation time was 30 s, and the procedure was carried out at room temperature. The $H_2O_2$ concentration was $5 \times 10^{-5}$ M, luminol concentration was 0.07 mM, $Na_2CO_3$ concentration was 1.18 mM, and the microperoxidase concentration was $3 \times 10^{-7}$ M. 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 concentrations of the samples were measured. The background chemiluminescence was evaluated with methanol as the sample. The percentage of the free radical scavenging activity was calculated as follows:

Free radical scavenging activity = 1-(chemiluminescence intensity with samples/background chemiluminescence intensity)

**Antilipoperoxidant activity.** Homogenates of the brains of young male Wistar albino rats weighing 150–200 g were prepared by the method of Fehér *et al.* (1985). The protein concentration of the brain homogenates was assayed by the method of Lowry *et al.* (1951).

Non-enzymatically induced lipid peroxidation was studied by incubating the protein suspension (1 mg/mL) in a medium of total volume 0.5 mL and containing 500 mM trismaleate buffer pH 6.8, 50 mM $KH_2PO_4$, different concentrations of ascorbic acid ($10^{-2}$M–$10^{-5}$ M), plus various concentrations of chervil and parsley extracts diluted in water (Blázovics and Fehér, 1992). The incubation temperature was 37 °C and 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 $mM^{-1}$ $cm^{-1}$ used.

**Statistical analysis.** The *in vitro* experimental results were mean ± SD of three parallel measurements. *p*-value <0.05 was regarded as significant.

## RESULTS

Figure 1 shows the results obtained with the methanol extracts tested with the chemiluminometric methods. Significant differences were found between the samples in the free radical scavenging activity. *Anthriscus cerefolium* scavenged more free radicals than *Petroselinum crispum*, while luteolin-7-O-glucoside was found to be the most effective at all concentrations. At a concentration of 0.001 mg/mL the percentage of the free radical activity ranged from 0% to 25% according to the methanol extract considered. Increasing the concentration to 0.005 mg/mL enhanced the activity, *Anthriscus cerefolium* scavenged more than 50% of the radicals present. Further increasing the concentration to 0.15 mg/mL resulted in all the samples quenching more than 82% of the radicals present.

Figures 2 and 3 show the antilipoperoxidant activities of the different concentrations of the samples on rat brain homogenates induced by ascorbic acid. Twenty minutes after addition of the homogenate, the control series showed a significant increase of the lipid peroxidation in terms of malondialdehyde production in the incubation medium. *Petroselinum crispum* demonstrated antilipoperoxidant activity in all concentrations of the sample and ascorbic acid, although at a concentration of 0.001 mg/mL the change was not significant. From the ascorbic acid concentration of $10^{-5}$M, the 0.01 and the

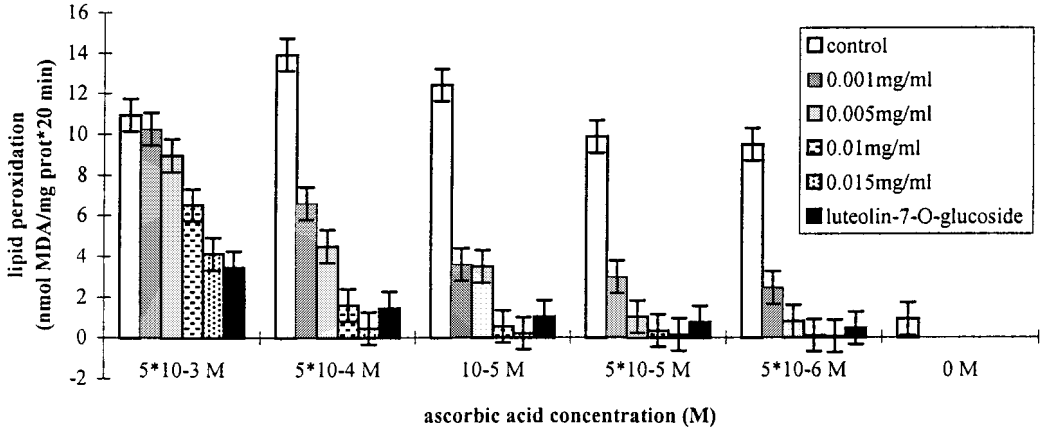


**Figure 2.** Effect of *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill. extract on ascorbic acid induced lipid peroxidation in rat brain homogenates. Results are mean ± SD of three parallel measurements, $p < 0.05$ compared with control.

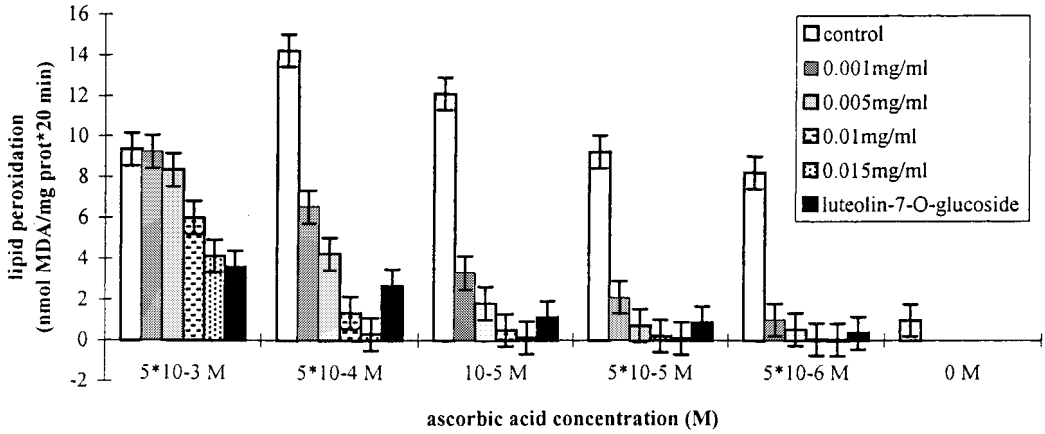


**Figure 3.** Effect of *Anthriscus cerefolium* L. Hoffm. extract on ascorbic acid induced lipid peroxidation in rat brain homogenates. Results are mean ± SD of five parallel measurements, $p < 0.05$ compared with control.

0.015 mg/mL concentration series inhibited the lipid peroxidation better than the reference luteolin-7-O-glucoside. *Anthriscus cerefolium* (Fig. 3) considerably protected the lipids from oxidative damage. The two highest concentrations of *Anthriscus cerefolium* samples proved to be more effective than the reference material luteolin-7-O-glucoside from an ascorbic acid concentration of $5 \times 10^{-4}$ M. The highest lipid peroxidation of the control series was measured at a concentration of $5 \times 10^{-4}$ M, whereas the lowest concentration of *Anthriscus cerefolium* sample reduced the malondialdehyde production by 50%.

## DISCUSSION

Analysis of the experimental results and the presumed compounds of the investigated crude plant extracts led to the following conclusions.

*Anthriscus cerefolium* and *Petroselinum crispum* had free radical scavenging and antilipoperoxidant effects on ascorbic acid induced lipid peroxidation *in vitro*. Our previous results testified that apiin is the main constituent in the methanol extracts (Fejes *et al.*, 1998). This suggests why both plants were found to be so effective. Comparing the chemiluminometric results with the lipid peroxidation studies, the following should be mentioned. While in the chemiluminometric experiments luteolin-7-O-glucoside was always more effective than the plant samples, in the lipid peroxidation measurements *Anthriscus cerefolium* and *Petroselinum crispum* samples proved (especially in higher concentration) to have a better activity than the reference material. This can be explained by the fact that in a plant extract there are several similar types of molecules, which can react synergistically, or enrich one another. Our investigation emphasizes that natural antioxidants can be a favourable choice as food additives, to preserve the quality of the food products against free radical attacks.

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