

# Antioxidant activity of medicinal plants used in phytotherapy

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**Key words:** *Sempervivum tectorum*, *Anthriscus cerefolium*, *Petroselinum crispum*, *Cichorium intybus*, *Helichrysum arenarium*, *Taraxacum officinale*, free radical scavenging activity, antioxidant activity.

**Summary:** Oxygen free radicals play an important role in the development of different disorders like inflammatory-immune injury, carcinogenesis, hepatic toxicity and atherosclerosis. The antioxidant role of a wide spectrum of natural products has been established. Flavonoids and other phenolic compounds (proanthocyanidins, rosmarinic acid, hydroxycinnamic derivatives, catechines, etc.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation.

We have studied the antioxidant activity as well as content and composition of natural phenolics in a series of medicinal plants with phytotherapeutical significance. Thus we determined the total phenol contents and studied the composition of flavonoids, polyphenols, phenolic acids of different vegetative and reproductive organs of medicinal plants: *Anthriscus cerefolium* (L.) Hoffm., *Petroselinum crispum* L., *Cichorium intybus* L., *Helichrysum arenarium* D.C., *Sempervivum tectorum* L., *Taraxacum officinale* Web.

Characteristic constituents in the various crude drugs were determined by chromatographic (TLC, HPLC) and spectroscopic (UV, UV-VIS) methods. The non specific scavenger activities of the medicinal plant extracts were studied by the chemiluminometric technique. The changes of chemiluminescence intensity of the  $H_2O_2/OH$ -luminol system at increasing concentrations of the  $H_2O_2/OH$  were measured. Inhibitory effects of selected standardized fractions from plants were tested on ascorbic acid induced lipid peroxidation in rat liver and homogenates.

The best correlation were established with total phenolics in some medicinal plants (*S. tectorum*, *T. officinale*) while activities in other cases seem to be influenced by flavonoids (*P. crispum*, *H. arenarium*, *A. cerefolium*) and by hydroxycinnamic derivatives (*C. intybus*).

## Introduction

Free radicals – highly reactive chemical species with one or more unpaired electrons – form in almost every cell of the body at an astonishing rate during normal oxidative metabolism. Environmental factors, such as UV light, ozone, and tobacco smoke contain free radicals or cause their formation. They react voraciously with almost every cellular component, and contribute to many types of pathology. For example, if their target is DNA, the likelihood of cancer increases; if it is low-density lipoprotein (LDL), the likelihood of atherosclerosis increases.

Antioxidant defense mechanisms counteract free radical formation and reactions. Some antioxidants are vitamins or

vitamin-forming compounds, which must be replenished through the diet: vitamin E, vitamin C, and the carotenoids, including beta-carotene. Others, such as glutathione, lipoic acid and ubiquinol, are manufactured by the body, but their levels can be bolstered through dietary supplementation. Each of these antioxidants has a specific area in which it is most effective; for example, vitamin E is the major chain-breaking antioxidant in membranes and blood lipoproteins, and vitamin C and the thiol antioxidants like glutathione are the major antioxidants in aqueous compartments.

It was thought that each antioxidant played its role in isolation from the others. But recent work indicates that there is a dynamic interplay among the systems. Nutritional

supplementation studies support this idea for the whole organism. Thus, there is emerging a picture of a complex interplay among the defense systems, with the various antioxidants cycles acting to prevent cell damage and disease.

If free radicals cause pathology and antioxidants neutralize free radicals, then increasing levels of antioxidants should decrease pathology. Combinations of antioxidants work better than separate antioxidants alone, epidemiological studies also support the idea that antioxidants are interdependent.

Hence, the evidence is quite strong for an oxidant-antioxidant balance in the body, which tips toward disease if oxidants predominate, but tips toward health if antioxidants predominate.

The natural antioxidant nutrients deserve attention as they offer the possibility to replace the optimal overall antioxidant status. The antioxidant role of a wide spectrum of natural products has been established. Medicinal plants - in this aspect - are undoubtedly promising as preventive agents. But a number of important questions must be addressed when formulating antioxidants either for therapeutic use or for addition to food. One of these questions is to consider the potential pro-oxidant actions of antioxidant supplements, antioxidant drug molecules and nutrient components.

The aim of the present work was to prove the phytotherapeutical significance of some official or popular medicinal plants on the base of their antioxidant activity due to their influence on pathological free radical reactions.

Experimental methods were planned and developed in order to measure the antioxidant, free radical scavenging, immunomodulant, membrane protecting activities and to monitor the cholesterol-, lipid peroxidation lowering capacity of plant extracts and enriched/isolated fractions. Complex phytochemical screening and analytical standardization was carried out considering the potential bioactive constituents. Characteristic constituents in the various crude drugs were determined by chromatographic and spectroscopic techniques. Model plants were selected in order to collect data for structure-activity relationships.

## Material and methods

### Materials

#### Plant materials

*Sempervivum tectorum* L. (Crassulaceae) plants were from the Botanical Garden in University of Horticulture, Budapest. The plant leaves were washed, excess water was removed, and leaves were then used for the preparation of extract following the method in SOTE Patent, No. 207657/1993. The resulted extract was lyophilized and used for the experiments. *Anthriscus cerefolium* L. Hoffm. was collected before the full flowering state from the hills of surrounding Budapest, Hungary. The dried herbs were ground before extractions. Dry samples of drugs were

purchased at drugstores and ground before extractions: *Petroselinum crispum* (Mill.) Nym ex A.W. Hill, *Taraxacum officinale* Web., *Cichorium intybus* L., *Helichrysum arenarium* (L.) Moench. The plant samples were extracted with water, methanol and in some cases (*Petroselinum crispum*, *Anthriscus cerefolium*) with n-hexane, chloroform, ethylacetate and methanol. The dried methanol extracts were separated on Sephadex LH-20 column using methanol as solvent. Methanol solutions were used in the chemiluminometric, while water solutions in the anti-lipoperoxidant experiments. Water extracts were lyophilized before use.

### Animals

Young male Wistar rats weighing 150–200 g were used. Animals were killed by decapitation, and the livers were removed. The microsomes were prepared by ultracentrifugation procedures (Blázovics et al., 1989).

### Hyperlipidaemic rats

Young male Wistar albino rats weighing 150–200 g were used. The animals were divided into three groups of 10 animals. The animals of group I were fed a normal LATI chow (Gödöllő, Hungary). Ten animals of group II, were fed an atherogenic diet consisting of 2,0% cholesterol, 20% sunflower oil and 0,5% cholic acid added to the control LATI chow. Ten animals of group III were fed the same lipid rich diet and treated with *Sempervivum tectorum* extract 2 g/kg body weight for 9 days, dissolved in the drinking water. The rats were killed by decapitation on day 9.

### Reagents

Xanthine oxidase (XO) was obtained from Boehringer (Mannheim), hypoxanthine (HT), desferrioxamine (DFO) and PBS from Sigma (St. Louis), 5,5-dimethyl-pyrroline-N-oxide (DMPO) from Shonan Analytic Center (Tokyo) and recombinant human superoxide dismutase (SOD) from Nikon Kayaku (Tokyo).

Cytochrome c and NADPH were obtained from Sigma (St. Louis), glucose-6-phosphate dehydrogenase and serum bovine albumin from Calbiochem AG (Lucerne, Switzerland).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) stable radical, microperoxidase, 5-amino-2,3-dihydro-1,4-phtalazinedion (luminol), cytochrome c and NADPH were obtained from Sigma (St. Louis), glucose-6-phosphate dehydrogenase, serum bovine albumin from Calbiochem AG (Lucerne). All other reagents were purchased from Reanal (Budapest).

### Biochemical measurements

Malondialdehyde (MDA) production was detected as an estimate of lipid peroxidation by the thiobarbituric acid test (Ottolenghi, 1959). A molar absorption coefficient  $E_{532}^{1\text{ cm}}$  of  $156\text{ mM}^{-1}\text{ cm}^{-1}$  was used.

The 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 50 mM Tris-maleate buffer pH 6,8, 1 mM  $\text{KH}_2\text{PO}_4$ ,  $5 \times 10^{-5}$  M  $\text{FeCl}_3$  and various concentrations of ascorbic acid and plant extract. The incubation temperature was 37 °C. Protein content of the preparation was determined by *Lowry et al.* (1951) using bovine serum albumin as a standard.

Natural scavenging capacity of lyophilized samples was detected by chemiluminometric method with Lumat LB 9051 luminometer, according to the method of *Blázovics* and co-workers (1999). Unstable free radicals, originated from  $\text{H}_2\text{O}_2$  in luminol-microperoxidase system via Fenton type reaction, catalysed the transformation of luminol into aminophthalic acid, when monochromatic light is emitted (expressed RLU: Relative Light Unit). In the presence of free radical scavenging molecules or compounds the emitted light is reduced. The  $I_{50}$  was determined and expressed as  $\mu\text{g}$ , that is the amount of the lyophilizate, which diminished emitted light of  $\text{H}_2\text{O}_2/\text{OH}$ -luminol-microperoxidase by 50%.

NADPH cytochrome c reductase activity was measured by *Jansson & Schenkman* (1977) at 550 nm in the presence and absence of NADPH and with different concentrations of dandelion extract.

Serum parameters were measured by a Hitachi 717 Analyzer. Free radical formation of rat liver homogenates was detected by a chemiluminometric method using a CLD-1 Medicor – Medilab luminometer. Luminescent light was measured with a sensitive photomultiplier. The electrical signals of the multiplier were processed by means of an MMT microprocessor system (*Zsinka et al.*, 1988).

## Phytochemical analysis

### Determination of polyphenol content

The polyphenol contents of the drugs, infusions and lyophilizates were measured according to the Hungarian Pharmacopoeia (Pharmacopoea Hungarica Editio VII, 1986) by spectrophotometric method at 750 nm, using pyrogallol as reference standard. This method is based on the formation of blue coloured products by redox reaction with Folin reagent.

### Flavonoid content determination

Flavonoid contents were determined spectrophotometrically in the samples according to the German Pharmacopoeia (*Deutsches Arzneibuch*, 1996) method, measuring the flavonoids in  $\text{AlCl}_3$ -complex form of purified ethyl acetate phase obtained after acid hydrolysis. Glycosides and aglycones were determined together in aglycone form.

### High-performance liquid chromatography

Chromatography was performed using a Spectra Physics HPLC system consisting of a P4000 quaternary gradient pump, a FOCUS scanning UV-VIS detector (Spectra-Physics Analytical, Fremont, CA, USA) in combination with a Rheodyne 7125 injector (20  $\mu\text{l}$  loop volume) and an IBM PS/2 computer. Analysis was performed on an Eurospher 100-C8 /5 $\mu\text{m}$ / reversed-phase Vertex column (250 $\times$ 4 mm

i.d.), with precolumn (5 $\times$ 4 mm i.d.) (Knauer, Berlin, Germany). The eluant was acetonitrile-tetrahydrofuran-30 mM citric acid in water (pH 3.0)-methanol (29:28:526:417, v/v/v/v). The flow-rate was 1 ml/min. Peaks were identified by co-chromatography with authentic standards and/or by diode-array detection (DAD).

## Statistical analysis

Results were assessed by one-way analysis of variance (ANOVA) and represent the mean  $\pm$ S.E.M. of three different measurements with two parallels.

## Results and discussion

*Sempervivum tectorum* (Figure 1) is a well known plant in folk medicine. The plant extract contains a notable quantity of low molecular mass antioxidative compounds (flavonol glycosides; approximately 20 different flavone and flavonol mono- and diglycosides, polyphenols, e.g. proanthocyanides, phenol carboxylic acid, polysaccharides, etc.) (*Kéry et al.*, 1992) (Figure 2).

The lyophilized extract of *Sempervivum tectorum* had a true superoxide scavenger activity in a cell free system. This suggests that this extract may also act as a direct scavenger of  $\text{O}_2^-$  in biological samples (Figure 3 and Table 1) (*Blázovics et al.*, 1993).

The extract inhibited the lipid peroxidation induced by ascorbic acid and  $\text{FeCl}_3$  *in vitro*, dose and time dependently.



Figure 1 *Sempervivum tectorum*

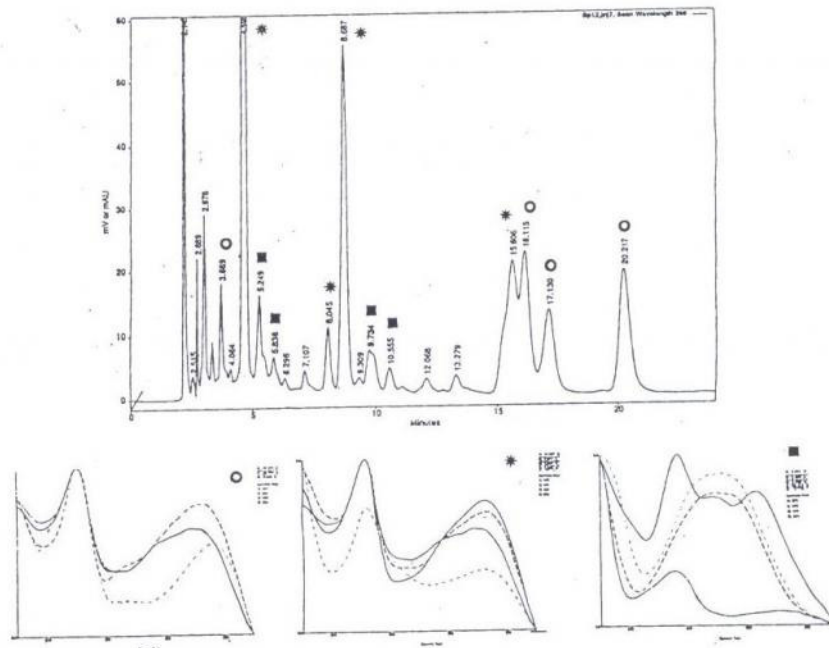


Figure 2 HPLC fingerprint and characteristic UV-spectra of Sempervivum fenoloids

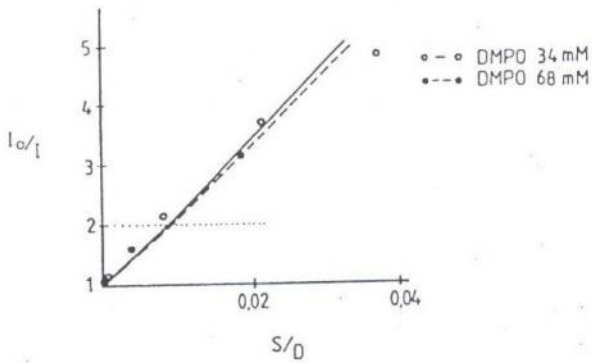


Figure 3 Superoxide scavenging activity of 'true scavenger'.  $I_0/I$  represents the ratio between the intensity of DMPO- $O_2^-$  spin adduct in the absence ( $I_0$ ) of the scavenger and that in the presence ( $I$ ) of the agent.  $S/D$  is the molar ratio between the scavenger (S) and DMPO, (D). The solid line with open circles and the dashed line with dots represent the SSA determined at 34 mM or 68 mM of DMPO, respectively. Values represent the mean of three measurements, correlation was greater than 0.985

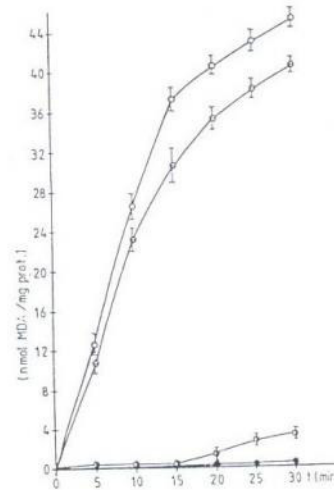


Figure 4 Dose-dependent effect of Sempervivum extract on ascorbic acid and  $Fe^{3+}$  induced lipid peroxidation on rat liver microsomal fraction.  $\circ-\circ$  control;  $\square-\square$  0.1 mg/mL StF1;  $\triangle-\triangle$  1.0 mg/mL StF1. Incubation time was 15 min. Ascorbic acid concentrations are shown.  $Fe^{3+}$  concentration was  $5 \times 10^{-5}$  M

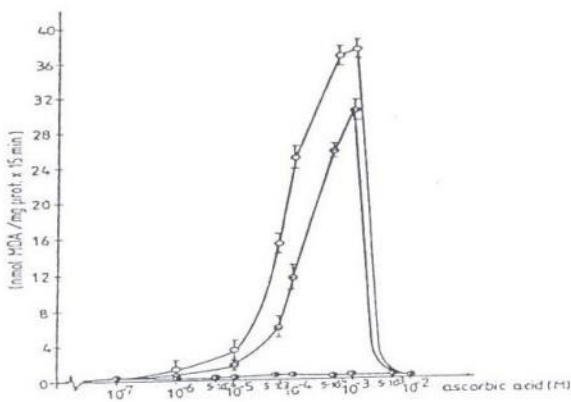


Figure 5 Time-dependent effect of Sempervivum extract on ascorbic acid and  $Fe^{3+}$  induced lipid peroxidation on rat liver microsomal fraction.  $\circ-\circ$  control;  $\square-\square$  0.1 mg/mL StF1;  $\triangle-\triangle$  1.0 mg/mL StF1;  $\bullet-\bullet$  10.0 mg/mL StF1. Ascorbic acid concentration was  $10^{-3}$  M. Incubation times were as shown.  $Fe^{3+}$  concentration was  $510^{-5}$

Table 1 Superoxide scavenger activity of StF1 determined by EPR spin trapping method

Sample	50% inhibition rate ( $I_{50}$ ) DMPO		Direct effect on the HT/XO system
	34.0 mM	68.0 mM	
StF1	0.25 mg	0.50 mg	no effect

These data indicate that the components of *Sempervivum* lyophilized extract can decrease the thiobarbituric acid reactive compounds liberated in the microsomal membrane fraction of rat liver (Figure 4 and Figure 5) (Blázovics et al., 1993).

Owing to the natural scavenger capacity of normal liver, the chemiluminescence of this system was decreased by control liver homogenates. The data suggest that the total scavenging capacity of homogenates of fatty livers is lower than that of controls. Although the specific enzyme activities could not be measured by the chemiluminometric technique, the change of light intensity during a given time interval indicated that *Sempervivum tectorum* treatment protected against  $H_2O_2/OH$ . This effect of prior extract treatment on the  $H_2O_2/OH$  scavenging function of fatty liver was detected in experiments in which the protein concentration was 1 mg/mL in a 5  $\mu$ L volume. The  $H_2O_2$  concentration was  $2.3 \times 10^{-6}$  M in the total volume. The significant biochemical and morphological changes caused by a lipid rich diet are reflected in the changes of values of serum parameters. In hyperlipidaemia the

suggests one possible way for reconsideration of the pharmaceutical activities. Free radical scavenging and membrane protective effects of *Petroselinum crispum* 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* 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

**Table 2** Changes in the serum parameters of rats treated with *Sempervivum tectorum* extract

Group (n=5)	ALP (U/L)	GOT (U/L)	GPT (U/L)	$\gamma$ GT (U/L)	TP (g/L)	GOT/GPT
I. Control	837.6 $\pm$ 100.0	183.6 $\pm$ 19.3	82.4 $\pm$ 13.8	0.8 $\pm$ 0.5	57.8 $\pm$ 1.1	2.22
II. Fat rich diet	2323.8 $\pm$ 254.3	186.7 $\pm$ 21.3	117.6 $\pm$ 7.6	1.5 $\pm$ 0.6	59.0 $\pm$ 2.8	1.67
III. Fat rich diet+ extract	1741.2 $\pm$ 311.7	177.6 $\pm$ 21.1	91.2 $\pm$ 14.4	1.6 $\pm$ 0.6	61.6 $\pm$ 2.2	1.94
p<0.05	I vs II s. II vs III s. I vs III s.	I vs II n.s. II vs III n.s. I vs III n.s.	I vs II s. II vs III s. I vs III n.s.	I vs II s. II vs. III n.s. I vs III s.	I vs II n.s. II vs III n.s. I vs III n.s.	

ALP, alkaline-phosphatase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase;  $\gamma$ GT, gamma-glutamyl transpeptidase;

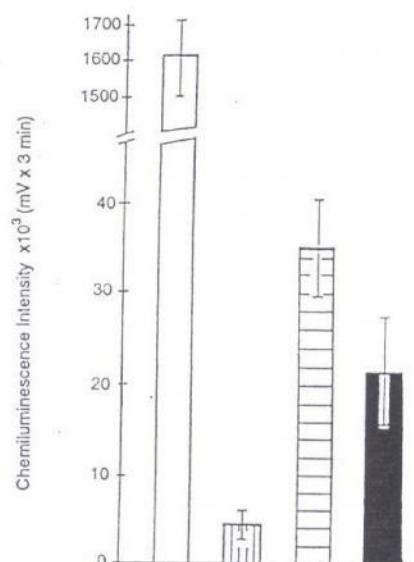
TP, total protein; GOT/GPT de Ritis quotient. Significance of difference, using student's Test from each group. S = p<0.05

serum level of ALP was increased by about three times, however, GOT and TP were not changed. The serum levels of GPT and gamma GT were significantly increased and the rate of GOT/GPT was decreased. After extract treatment the ALP and GPT serum levels were changed and the rate of GOT/GPT was also altered favourably (Table 2).

We demonstrated the role of free radical reactions and lipid peroxidations in the pathomechanism of fatty liver. If the defence mechanism of liver cells are badly damaged, the total free radical scavenger capacity decreases. In the test system the chemiluminescence of the  $H_2O_2/OH$ -luminol system was very high (Figure 6) (Blázovics et al., 1994).

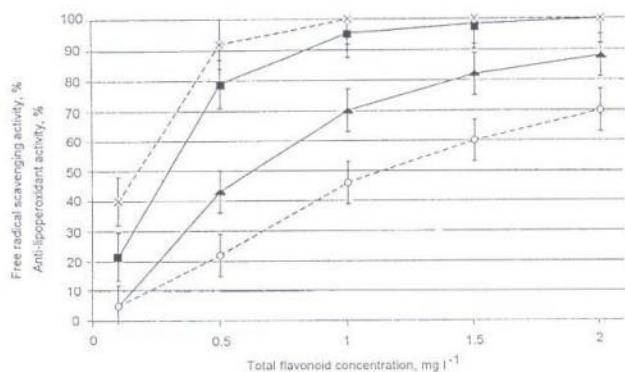
Parsley (*Petroselinum crispum*) has been used as a popular spice and vegetable. The seeds have a strong diuretic activity due to its high essential oil content. The leaves are widely used as a spice. The characteristic constituents are essential oil (apiol, miriszticin), flavonoids (apiin, luteolin-,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

Since the phytotherapeutic effects of parsley have not yet been fully confirmed and analysed this investigation



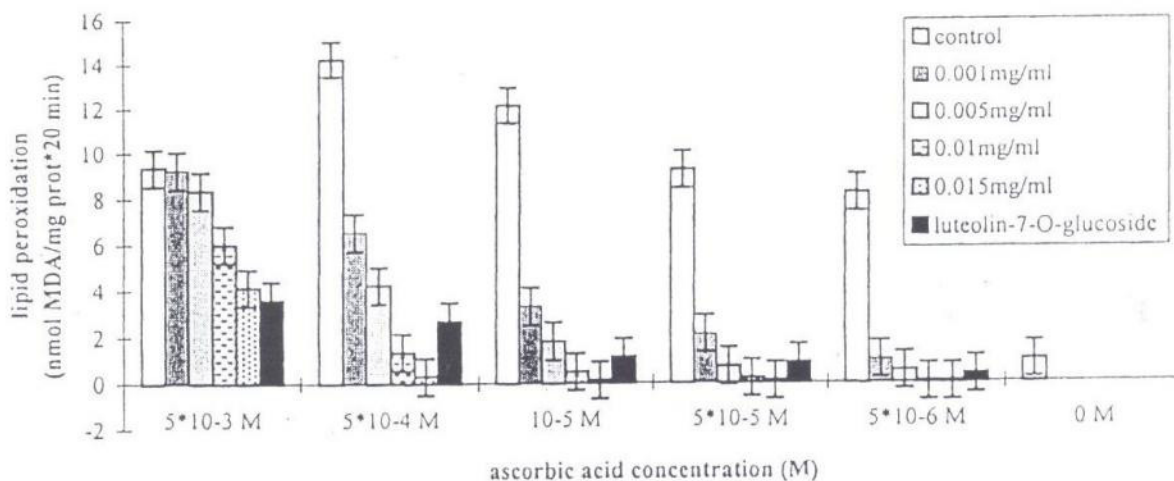
**Figure 6** Effect of *Sempervivum tectorum* extract on natural scavenger capacity of hyperlipidaemic rats in  $H_2O_2/OH$ -luminol system. The measuring time was 3 min and the temperature was 25 °C during experiments. The total volume was 1150  $\mu$ L. □,  $H_2O_2/OH$ ; ▨, normo-lipidaemia; ■, hyperlipidaemia + *Sempervivum tectorum*

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.



**Figure 7** 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  $\pm$  S.D. of three parallel measurements.  $\blacktriangle$  : free radical scavenging activity of apiin;  $\blacksquare$  : free radical scavenging activity of the herb;  $\circ$  : anti-lipoperoxidant activity of apiin;  $\times$  : anti-lipoperoxidant activity of the herb

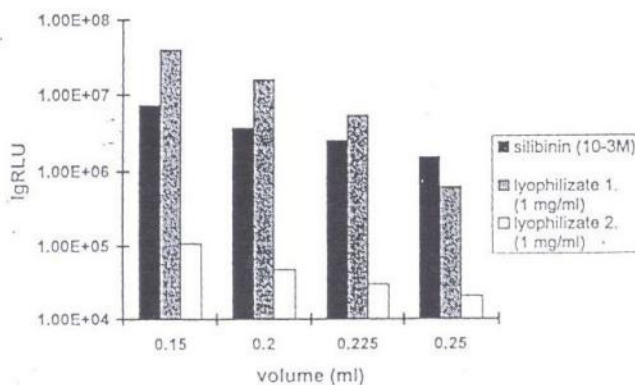
Both conclusions can be explained by the fact that in a plant extract there are several similar types of molecules, which can react synergically, or enrich one another. This is the reason why apiin and the almost pure apiin containing S4 sample showed lower activity in the biological environment. Probably 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. Studies are in progress to analyse the



**Figure 9** Effect of *Anthriscus cerefolium* L. Hoffm. extract on ascorbic acid induced lipid peroxidation in rat brain homogenates. Results are mean  $\pm$  SD of five parallel measurements,  $p < 0.05$  compared with control

synergetic effects (Figure 7, Figure 8) (Fejes et al., 2000).

Chervil, *Anthriscus cerefolium* L. (Hoffm.) belonging to the Apiaceae 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 (Brenness, 1989). Characteristic constituents of the herb are flavonoids



**Figure 8** 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 chromatography, compared to apiin. Results are mean  $\pm$  S.D. of three parallel measurements.  $\blacksquare$  : free radical scavenging activity;  $\square$  : anti-lipoperoxidant activity

(apiin, luteolin-glycosides) (Tozaburo & Masao, 1979), and essential oil (methyl-chavicol = estragole, 1-allyl-2,4-dimethoxybenzene) (Zwaving et al., 1970; Simándi et al., 1996).

*Anthriscus cerefolium* 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 it was 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* sample 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 (Figure 9) (Fejes et al., 2000).

The choleric, hepatoprotective and detoxifying activities of the inflorescence of *H. arenarium* has been known for a long time from herbal medicine in Hungary. While the first therapeutic uses of the plant were based on folk medicine, recent in vitro and in vivo studies also proved its choleric (Dombrowicz et al., 1994) and hepatoprotective (Skakun & Stepanov, 1988) properties.

A number of different components have been found (flavonoids, coumarines, phtalides,  $\alpha$ -pyron derivatives, terpenoids, essential oils, volatile and fatty acids) (Vrkoc et al., 1971; Roth & Schmid, 1976; Derkach et al., 1986), among which the most important are the flavonoids (Prokopenko et al., 1972; Vrkoc et al., 1973).

On the basis of our experiments, it could be concluded that the bioactive components of *Helichrysi flos* can act as primary and secondary antioxidants, scavenge free radicals and therefore inhibit the lipid peroxidation, and may have beneficial effect on prevention of liver and gallbladder diseases (Blázovics et al., 1996; Hollman & Katan, 1998) where reactive oxygen species are involved. Antioxidant properties of its phenolic and flavonoid compounds can be at the origin of these effects, but further in vivo experiments are planned to verify relation between chemical composition and antioxidant activity (Figure 10) (Czinner et al., 2000).

*Taraxacum officinale* is a common plant in temperate climates, particularly in Western Europe, where it inhabits fields, roadsides and waste grounds (Lowell & Rowan, 1991). The drug is collected from both wild and cultivated plants (Figure 11).

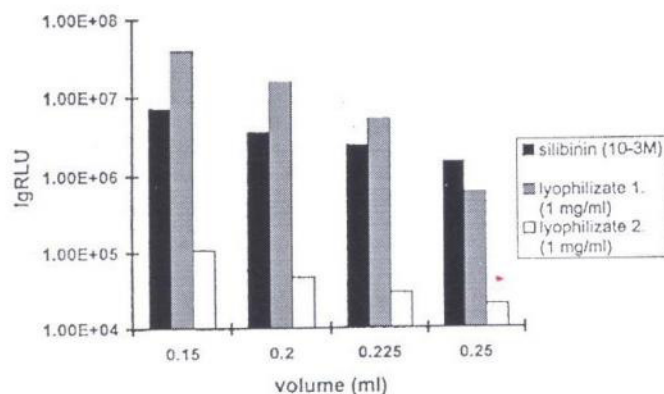


Figure 10 Total scavenger capacity of the *Helichrysum* samples in  $H_2O_2/OH^-$  – luminol-microperoxidase system (expressed as Relative Light Units (RLU)). Standard light, 13 094 841.5 $\pm$ 383 102 RLU. ■, silibinin; ▨, lyophilizate 1; □, lyophilizate 2



Figure 11 *Taraxacum officinale*

The main constituents of dandelion are: sesquiterpene lactones (germacranolides, eudesmanolides), triterpens including pentacyclic alcohols (taraxol, taraxerol,  $\beta$ -amirin, taraxasterol, arnidiol, farnidiol), phytosterols (sitosterol, stigmasterol), flavonoids (apigenin, luteolin 7-glycosides), acids (caffeic acids, *p*-hydroxyphenylacetic acid, chlorogenic acid, linoleic acid, oleic acid etc.), sugars (fructose, glucose, sucrose), choline, inulin, pectin. The high potassium and vitamin (A, B, C, D) contents are also worth mentioning.

Dandelions have long been used in herbal medicine for their choleric, diuretic, antirheumatic, anti-inflammatory, laxative, appetite-stimulating properties for treating liver and gallbladder disorders, digestive complaints (lack of appetite, feeling of distension or flatulence), arthritic and rheumatic diseases as well as eczema and other skin conditions.

Flavonoids can inhibit the activity of enzymes such as lipoxygenase, cyclooxygenase, xantin oxidase, phospholipase A2, protein kinases (Cao et al., 1997; Hollman & Katan, 1998). Polyphenols in general can influence the lipid peroxidation process in the same way, and can also act as direct scavenger molecules, therefore it can be supposed that they have beneficial, multifactorial effect on the liver microsomal membranes.

Our results demonstrated dandelion's beneficial effect on lipid peroxidation in liver microsomes, in addition its natural extracts were able to stimulate NADPH cytochrome P450 reductase activity (Figure 12) (Hagymási et al., 2000).

The extracts of folium and root showed H-donating ability, reducing power property and natural scavenging activity in  $H_2O_2/OH^-$ -luminol-microperoxidase system, depending on the concentration.

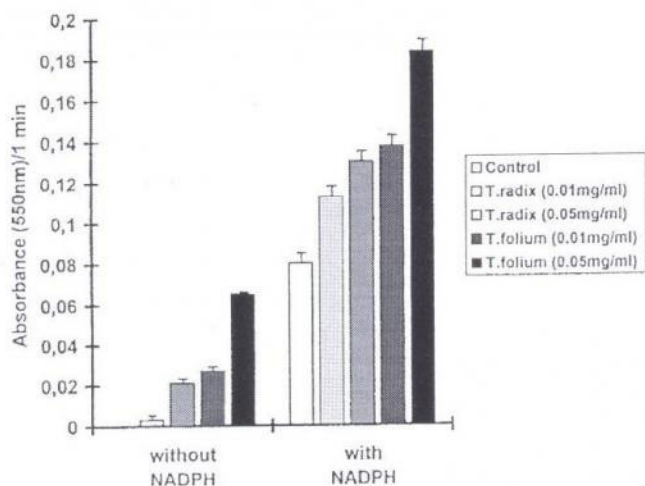


Figure 12 Activity of NADPH-cytochrome P-450 reductase in rat liver microsomal fraction without and with NADPH

The folium extract with higher polyphenol and flavonoid content proved to be more effective in all three in vitro systems.

Therefore active compounds of dandelion with primary and secondary antioxidant as well as scavenging properties can preserve the structure of membranes and protect against secondary lipid peroxidation, but further in vivo investigations are needed to confirm its existence in human nutrition as a natural source of antioxidants (Hagymási et al., 2000).

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