Antioxidant activity of some herbal extracts in rapeseed and sunflower oils

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Summary
Antioxidant and prooxidant properties of natural antioxidant concentrates with high contents of flavonoids, carotenoids and phenolic acids were studied. Antioxidant concentrates from marigold (*Calendula officinalis* L.), basil (*Ocimum basilicum* L.), self-heal (*Prunella vulgaris* L.) and wool mullein (*Verbascum densiflorum* L.) were added to sunflower and rapeseed oil, respectively, at concentrations ranging from 2 mg.kg^{-1} to 200 mg.kg^{-1} oil. The Rancimat assay was used for the optimization of antioxidant doses of individual plant concentrates. The most effective antioxidant in both sunflower and rapeseed oil was the marigold concentrate (optimal dose was 10 mg.kg^{-1} and 30 mg.kg^{-1}, respectively). Basil concentrate possessed an antioxidative effect comparable with the antioxidative effect of marigold concentrate - the optimal dose was 2 mg.kg^{-1}.

Keywords
plant extracts; vegetable oils; antioxidant; Rancimat assay

The primary cause of lipid oxidation is the action of free radicals, which initiate degradation of unsaturated fat. Various synthetic antioxidants have been approved and routinely used as food protectants. However, increasing public demand for the replacement of synthetic antioxidants by natural ones has initiated intense research activity that resulted in the screening of a wide variety of plant extracts and stimulated efforts to discover effective preservatives for a wide range of foodstuffs [1-7]. Medicinal plants contains many types of effective antioxidants, mostly phenolic acids, flavonoids and carotenoids [8-12]. Compounds with antioxidant activity may behave as prooxidants under certain conditions or in high concentrations. Prooxidant activity can accelerate oxidative damage to different compounds [13-19]. Potential antioxidant should therefore be tested for prooxidant activity as well.

The objective of this study was to measure the antioxidant and prooxidant activities of selected plant extracts by Rancimat assay and to determine optimal doses of these extracts for use in sunflower and rapeseed oil.

MATERIALS AND METHODS

Four selected plants - marigold (*Calendula officinalis* L. - petals), basil (*Ocimum basilicum* L. - leaves), self-heal (*Prunella vulgaris* L. - leaves and flowers) and wool mullein (*Verbascum densiflorum* L. - flowers) were dried in a ventilated oven at (30 ± 2) °C. Dried plants were ground (max. particle size 1 mm) and 2 g were extracted by one-step extraction with 100 ml of 60% ethanol at 60 °C for 1 h in a whirling thermostate (MLW, Baureime, Germany). After cooling, the extracts were filtered through filter paper (Filtrak No. 390, Spezialpapierfabrik, Niederschlag, Germany). The solvent was evaporated in a vacuum rotary evaporator to the volume 25 ml. All extracts were examined for the antioxidant activity using the individual methods. The measurements were done in triplicates.

Sunflower oil (peroxide number 0.56 mmol.kg^{-1}, free fatty acids 0.057%) and rapeseed oil (peroxide number 0.53 mmol.kg^{-1}, free fatty acids 0.046%) were the products of Palma-Tumys (Bratislava, Slovakia). The 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) was purchased from Aldrich (Steinheim, Germany). For measurements, the stock solution

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of 0.025 g.l^{-1} DPPH in methanol was used. The Folin-Ciocalteu agent was from Merck (Darmstadt, Germany). Tannic acid from Aldrich (Steinheim, Germany) was used as 0.03% aqueous solution. Quercetin, β-carotene and BHT were purchased from Sigma (Steinheim, Germany).

Evaluation of the oxidation stability

The oxidation stability of oils was determined using the Rancimat assay (Metrohm 743 Rancimat instrument, Herisau, Switzerland) according to Verleyen [20]. Rapeseed or sunflower oil were used as the lipid substrate to evaluate ability of the extracts to inhibit lipid oxidation. 10–100 μl of plant extract were added to 3 g of oil. The experiment was carried out at 100 °C with air flow 20 l.h^{-1}. The volatile acids formed by oxidation were carried by the effluent air and trapped in vessels containing 50 ml of redistilled water. The conductivity in these vessels was measured and recorded continuously. The induction period (*IP*) is the time elapsed until the inflection point of the conductivity vs. time curve recorded by the Rancimat. The inhibitory activity was expressed as protection factor (*PF* - the ratio of induction periods determined in the presence or absence of antioxidant) used for optimization of antioxidant addition and as the induction period (*IP*) used as indicator of stability during storage of oils. The values are averages of measurements done in triplicate, the standard deviation was also determined.

Evaluation of antioxidant activity by phosphomolybdenum method

The total antioxidant activity was evaluated by the method of Prieto [21]. An aliquot of 0.1 ml of sample solution was combined with 1 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 80 °C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution was measured at 700 nm against blank using spectrophotometer UV-1601 (Shimadzu, Tokyo, Japan). Reductive power of compounds (RP_{AA}) expressed as quantity of ascorbic acid necessary to achieve the same effect in μg.ml^{-1} was calculated using the equation:

$$RP_{AA} = (A_{705\text{nm}} - 0.0011) / 0.00236$$

Evaluation of antiradical activity by DPPH radical

The procedure of Brand-Williams [22, 23] was adapted as follows: 3.9 ml of the DPPH solution in methanol (0.025 g.l^{-1}) were transferred into 1 cm cuvette and the absorbance value A_0 was measured against blank at 515 nm using spectrophotometer UV-1601 (Shimadzu, Tokyo, Japan). The plant extract was added into the cuvette containing the DPPH solution. After stirring, the decrease in absorbance was measured immediately at 1 s intervals during 600 s. The antiradical activity of individual extracts under the steady-state conditions was expressed as the percentage of inhibition of DPPH using the equation:

$$\text{DPPH}\ [\%] = [c(\text{DPPH})_t / c(\text{DPPH})_0] \times 100$$

Spectrophotometric determination of flavonoids

The total flavonoid content was determined by the method of Chang et al. [24] based on the principle that aluminium chloride forms acid-resistant complexes with the C-4 keto group and either the C-3 or the C-5 hydroxyl group of flavones and flavonols. Quercetin was used as the reference flavonoid for making calibration curve. The absorbance was measured at 415 nm. Results were expressed as quercetin equivalents (mg.l^{-1}).

Spectrophotometric determination of carotenoids

The total carotenoid content was determined spectrophotometrically by the Britton method at λ_{max} 445 nm [25]. Carotenoid content was calculated as mg of β-carotene per liter using the extinction coefficient $A^{1\%}_{1\,cm}$ = 2620 (determined by dissolution of known quantities of β-carotene standard).

HPLC determination of rosmarinic acid

The rosmarinic acid in antioxidant concentrates was determined by HPLC with UV/VIS detector at 325 and 294 nm (LC 10 ADVP pump with gradient FCV 10 ALVP, detector UV-VIS SP 10 AVVP, Shimadzu, Tokyo, Japan). Rosmarinic acid was separated on Reprosil 100 C18 column, 5 μm, 250 × 4 mm (Watrex, San Francisco, USA), flow rate 0.5 ml.min^{-1}; mobile phase A: 0.1% trifluoracetic acid, B: acetonitrile, gradient: 0–5 min 10% B, 5–20 min 35% B, 20–40 min 70% B, 40–41 min 90% B, 41–42 min 50% B, 42–43 min 25% B, 43–44 min 5% B, 45 min 100% A.

RESULTS AND DISCUSSION

Characteristics of the concentrates used are summarised in Tab. 1. The highest antiradical activity exhibited balm and self-heal concentrates. These concentrates contain mainly rosmarinic and caffeic acid [1-3, 10, 26, 27] as active substances. The concentrate from basil contains flavonoids and carotenoids in addition to fenolic acids [9-11,

Tab. 1. Characterization of plant extracts.

Plant	RPKA [μg.ml^{-1}]	DPPH [% inhibition]	rosmarinic acid [mg.l^{-1}]	flavonoids [mg.l^{-1}]	carotenoids [mg.l^{-1}]
basil	763	85.12	252	106	77
wool mullein	2550	48.92	–	568	62
self-heal	650	72.60	48	67	25
calendula	2502	58.19	–	287	415

Tab. 2. Protection factor of sunflower oil.

Dose [mg.kg^{-1} oil]	Protection factor (*PF*)				
	BHT	basil	self-heal	calendula	wool mullein
2	1.00	**1.34**	1.04	1.27	1.01
5	1.03	1.31	1.14	1.32	1.04
10	1.05	1.26	1.27	**1.46**	1.08
15	1.10	1.11	**1.32**	1.41	1.13
20	1.14	1.01	1.24	1.38	1.16
30	1.19	0.94	1.18	1.25	**1.18**
40	1.25	0.89	1.11	1.17	1.12
60	**1.33**	0.75	1.11	1.12	1.05
100	1.27	0.63	1.09	1.08	0.88
200	1.22	0.54	0.92	0.95	0.78

Tab. 3. Protection factor of rapeseed oil.

Dose [mg.kg^{-1} oil]	Protection factor (*PF*)				
	BHT	basil	self-heal	calendula	wool mullein
2	1.00	**1.41**	1.05	1.10	1.04
5	1.02	1.36	1.17	1.19	1.07
10	1.05	1.33	1.24	1.24	1.12
15	1.08	1.24	1.28	1.31	1.17
20	1.13	1.15	1.33	1.38	1.22
30	1.17	1.08	**1.36**	**1.43**	**1.26**
40	1.23	1.03	1.29	1.40	1.25
60	1.29	0.97	1.21	1.32	1.18
100	**1.32**	0.91	1.14	1.13	1.09
200	1.26	0.89	1.08	0.97	1.01

26]. Phytochemical studies indicate that *Prunella vulgaris* further contains oleanolic, betulinic, ursolic acids, triterpenoids, flavonoids and other active compounds [28]. The highest reduction activity exhibited concentrates of wool mullein and marigold, which contain mainly flavonoids and carotenoids [4, 8, 29-32].

The highest protection effect in sunflower oil was exhibited by the basil concentrate at the dose of 2 mg.kg^{-1} (Tab. 2). Increase of the dose led to the decrease of the protection factor. The optimum dose of concentrates was 15 mg.kg^{-1} for the self-heal, 10 mg.kg^{-1} for the marigold and 30 mg.kg^{-1} for wool mullein. All concentrates exhibited prooxidant effect at doses higher than 30 mg.kg^{-1}. For the synthetic antioxidant BHT a dose of 60 mg.kg^{-1} was necessary to achieve the maximal protection effect.

By addition of basil concentrate at a dose of 2 mg.kg^{-1} to rapeseed oil a protection factor of 1.41 was achieved. Higher doses had smaller antioxidant, sometimes even prooxidant effects. The optimum doses of other concentrates were 30 mg.kg^{-1}. The highest protection factor exhibited the marigold concentrate (*PF* 1.43), followed by self-heal (*PF* 1.36). The lowest protection factor was achieved by addition of wool mullein concentrate (*PF* 1.26), in spite of its highest reduction power (Tab. 3).

From the above results it follows that, based on the reduction and antiradical properties of individual concentrates and their composition (Tab. 1), it is not possible to determine application doses of the individual concentrates. The possible reason of this fact may be the synergic effects of individual active compounds.

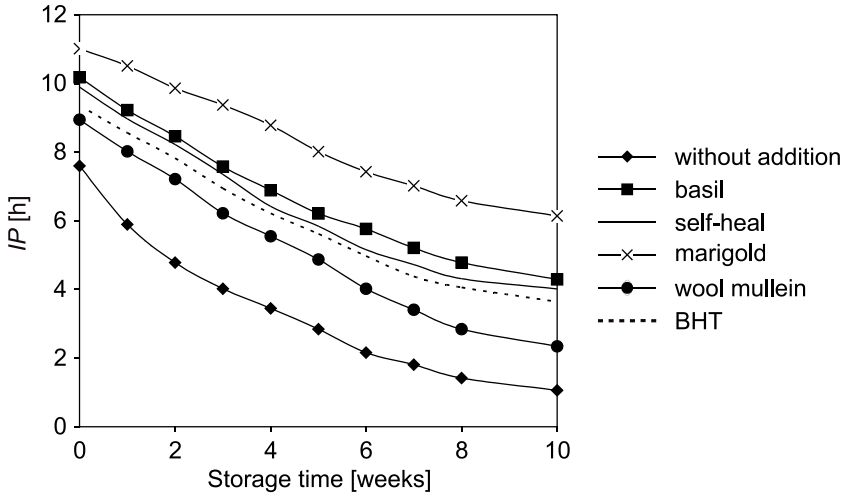

Fig. 1. Dependence of induction periode (*IP*) on storage time - sunflower oil.
Addition of extracts: basil - 2 mg.kg^{-1}, self-heal - 15 mg.kg^{-1}, marigold - 10 mg.kg^{-1}, wool mullein - 30 mg.kg^{-1}.

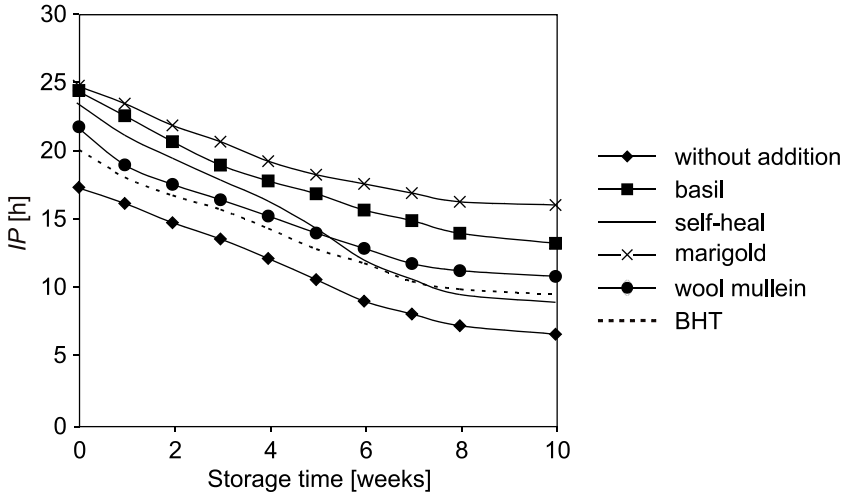

Fig. 2. Dependence of induction periode (IP) on storage time - rapeseed oil.
Addition of extracts: basil - 2 mg.kg^{-1}, self-heal - 30 mg.kg^{-1}, marigold - 30 mg.kg^{-1}, wool mullein - 30 mg.kg^{-1}.

In the rapeseed oil a BHT dose of 100 mg.kg^{-1} was necessary to achieve stabilisation effect comparable to any plant concentrate. The protection factor greater than 1 indicates that the concentrate inhibits oxidative rancidity.

Optimal doses of the individual concentrates and of BHT were added to the oils and the stability of oils was monitored during storage at (20 ± 3) °C in the dark. The highest protection effect during storage of sunflower oil exhibited the marigold concentrate. Basil and self-heal concentrates exhibited comparable stabilisation effect in sunflower oil after 10 weeks of storage (Fig. 1). The effect of BHT was lower by 45% compared to that of marigold concentrate, which was the best preservative tested.

In the case of rapeseed oil, the highest stability during storage was observed for samples containing marigold concentrate, as well as in the case of sunflower oil. Toward the end of the storage period, wool mullein concentrate was more effective than self-heal concentrate, in spite of the fact, that the former had lower initial stabilisation influence (Fig. 2).

The addition of marigold concentrate to rapeseed and sunflower oil extended the induction period of their oxidation. The induction period for rapeseed oil was (16.01 ± 0.17) h and (6.14 ± 0.12) h for sunflower oil. In the control experiment, this induction period was (6.54 ± 0.12) h for rapeseed oil and (1.06 ± 0.03) h for sunflower oil after 10 weeks of storage.

CONCLUSION

Influence of plant concentrates on the stability of sunflower and rapeseed oils during storage by the Rancimat assay was examined. The best protection effect during storage of both oils was shown by the marigold concentrate. In the sunflower oil the optimum dose was found to be 10 $mg.kg^{-1}$, whereas in rapeseed oil a dose of 30 $mg.kg^{-1}$ was necessary. These doses are lower than the necessary doses of the synthetic antioxidant BHT. Thus the studied natural concentrates can be recommended as suitable substitutes for synthetic antioxidants.

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REFERENCES

1. Marinova, E. M. - Yanishelieva, N. V.: Antioxidant activity of extracts from selected species of the family *Lamiaceae* in sunflower oil. Food Chemistry, *58*, 1997, pp. 245-248.
2. Psotová, J. - Kolář, M. - Soušek, J. - Švagera, Z. - Vičar, J. - Ulrichová, J.: Biological activities of *Prunella vulgaris* extract. Phytotherapy Research, *17*, 2003, pp. 1082-1087.
3. Liu, F. - Ng, T. B.: Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Science, *66*, 2000, pp. 725-735.
4. Aligiannis, N. - Mitaku, S. - Tsitsa-Tsardis, E. - Harvala, C. - Tsaknis, I. - Lalas, S. - Haroutounian, S.: Methanolic extract of *Verbascum marcrurum* as a source of natural preservatives against oxidative rancidity. Journal of Agriculture and Food Chemistry, *51*, 2003, pp. 7308-7312.
5. Schwarz, K. - Bertelsen, G. - Nissen, L. R. - Gardner, P.: Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assay based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. European Food Research Technology, *212*, 2003, pp. 319-328.
6. Milauskas, G. - Venskutonis, P. R. - Beek, T. A.: Screening of radical activity of some medicinal and aromatic plant extracts. Food Chemistry, *85*, 2004, pp. 231-237.
7. Sekretár, S. - Schmidt, Š. - Vajdák, M.: Screening of antioxidative and antimicrobial activity of selected plant extracts. Chemické Listy, *98*, 2004, pp. 685-686.
8. Cordova, C. A. - Siquera, I. R. - Netto, C. A.: Protective properties of butanolic extract of the *Calendula officinalis* L. (marigold) against lipid peroxidation of rat liver microsomes and action as free radical scavenger. Redox Report, *7*, 2002, pp. 95-102.
9. Proestos, C. - Boziaris, I. S. - Nychas, G.-J. E. - Komaitis, M.: Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobioal activity. Food Chemistry, *95*, 2006, pp. 664-671.
10. Jayasinghe, Ch. - Goton, N. - Aoki, T. - Wada, S.: Phenolics composition and antioxidant activity of sweet basil. Journal of Agriculture and Food Chemistry, *51*, 2003, pp. 4442-4449.
11. Yanishlieva, N. - Marinova, E.: Natural antioxidants in lipid oxidation. Bulgarian Chemical Communications, *35*, 2003, pp. 79-91.
12. Chen, J. H. - Ho, Ch-T.: Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. Journal of Agriculture and Food Chemistry, *45*, 1997, pp. 2374-2378.
13. Cao, G. - Sofic, E. - Prior, R. L.: Antioxidant and prooxidant behaviour of flavonoids: structure-activity relationships. Free Radical Biology and Medicine, *22*, 1997, pp. 749-760.
14. Heinen, C. G. - Haenen, G. R. - Oostveen, R. M. - Stalpers, E. M. - Bast, A.: Protective of flavonoids against lipid peroxidation: the structure activity relationship revisited. Free Radical Research, *36*, 2002, pp. 575-581.
15. Amic, D. - Amic-Dragivic, D. - Bešlo, D. - Trinajstic, N.: Structure-radical scavenging relationship of flavonoids. Croatia Chemica Acta, *76*, 2003, pp. 55-61.
16. Subario, A. - Morita, N.: Prooxidant activity of lutein and its dimyristate esters in corn triacylglyceride. Food Chemistry, *81*, 2003, pp. 97-102.
17. Young, A. J. - Lowe, G. M.: Antioxidant and prooxidant properties of carotenoids. Archives of Biochemistry and Biophysics, *385*, 2001, pp. 20-27.
18. Fukumoto, L. R. - Maya, G.: Assesing antioxidant and prooxidant activities of phenolic compounds. Journal of Agriculture and Food Chemistry, *48*, 2000, pp. 3597-3604.
19. Ferancová, A. - Heilerová, Ľ. - Korgová, E. - Šilhár, S. - Štepánek, I. - Labuda, J.: Anti/pro-oxidative properties of selected standard chemicals and tea extracts investigated by DNA-based electrochemical biosensor. European Food Research Technology, *219*, 2004, pp. 416-420.
20. Verleyen, T. - Dyck, S. - Adams, C. A.: Use of accelerated oxidation texts to evaluate antioxidant activity. Lipid technology, *16*, 2004, pp. 39-41.
21. Prietto, P. - Pineda, M. - Aquilar, M.: Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specification application to the determination of vitamin E. Analytical Biochemistry, *269*, 1999, pp. 337-341.
22. Brand-Williams, W. - Cuvelier, M. E. - Berset, C.: Use of a free radical method to evaluate antioxidant activities. Lebensmittel Wissenschaften unter Technology, *28*, 1995, pp. 25-30.
23. Sánchez-Moreno, C. - Larrauri, J. - Saura-Calixto, F.: A procedure to measure the antiradical efficiency

of polyphenols. Journal of Agriculture and Food Chemistry, *76*, 1998, pp. 270-276.
24. Chang, C. C. - Yang, M. Y. - Wen, H. M. - Chern, J. C.: Estimation of total flavonoids content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10, 2002, pp. 178-182.
25. Britton, G.: General carotenoid methods. Methods in Enzymology, *111*, 1985, pp. 113-149.
26. Javanmardi, J. - Khalighi, A. - Kashi, A. - Bais, H. P. - Vivanco, J. M.: Chemical characterisation on basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. Journal of Agriculture and Food Chemistry, *50*, 2002, pp. 5878-5883.
27. Holzmanová, V.: Rosmarinic acid and its biological activity. Chemické Listy, *90*, 1996, pp. 486-496.
28. Škottová, N. - Večera, R. - Váňa, P. - Ulrichová, J. - Walterová, D.: Antioxidant activities of polyphenolic extract from *Prunella vulgaris* in rat. Atherosclerosis, *2*, 2001, pp. 116-117.
29. Pietto, P. - Bruno, A. - Mauri, P. - Rava, A.: Separation of flavonol-2-O-glycosides from *Calendula officinalis* and *Sambucus nigra* by HPLC and micellar electrocinetic capillary chromatography. Journal of Chromatography, *28*, 1992, pp. 165-170.
30. Bakó, E. - Deli, J. - Tóth, G.: HPLC study on the carotenoid composition of *Calendula* products. Biochemistry and Biophysical Methods, *53*, 2002, pp. 241-250.
31. Piccaglia, R. - Marotti, M.: Effects of harvesting date and climate on the flavonoid and carotenoid contents of marigold (*Calendula officinalis* L.). Flavour and Fragrance Journal, *12*, 1997, pp. 85-90.
32. Klimek, B.: Hydroxycinnamoyl ester glycosides and saponins from flowers of *Verbascum phomoides*. Phytochemistry, *43*, 1996, pp. 1281-1284.

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