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Screening pharmaceutical preparations containing extracts of turmeric rhizome, artichoke leaf, devil's claw root and garlic or salmon oil for antioxidant capacity

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Abstract

Pharmaceutical preparations derived from natural sources such as vegetables often contain compounds that contribute to the antioxidant defence system and apparently play a role in the protection against degenerative diseases. In the present study, commercial preparations containing extracts of turmeric, artichoke, devil's claw and garlic or salmon oil were investigated. The products were divided into fractions of different polarity, and their antioxidant activity was determined using the Trolox equivalent antioxidant capacity (TEAC) assay. This test is based on the efficacy of the test material to scavenge 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) derived radicals. Total phenols were determined in all fractions as well as specific carotenoids in the most lipophilic fraction to assess their contribution to the antioxidant activity. For comparison, the radical scavenging effect of selected constituents of the extracts such as curcumin, luteolin, kaempferol, chlorogenic acid, harpagoside, β-carotene and α-tocopherol was investigated and compared with that of Trolox. Curcumin, luteolin, kaempferol, chlorogenic acid and β-carotene showed an antioxidant activity superior to Trolox in the TEAC assay; harpagoside was barely active. All fractions of the turmeric extract preparation exhibited pronounced antioxidant activity, which was assigned to the presence of curcumin and other polyphenols. The antioxidant activity corresponding to the artichoke leaf extract was higher in the aqueous fractions than in the lipophilic fractions. Similarly, devil's claw extract was particularly rich in water-soluble antioxidants. Harpagoside, a major compound in devil's claw, did not contribute significantly to its antioxidant activity. The antioxidant capacity of the garlic preparation was poor in the TEAC assay. That of salmon oil was mainly attributed to vitamin E, which is added to the product for stabilization. In all test preparations, the antioxidant activity was significantly correlated with the content of total phenolic compounds.

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Introduction

Reactive oxygen species, which are generated in exogenous and endogenous processes, are involved in the pathogenesis of several degenerative diseases, such as atherosclerosis, cancer and cataract, and play a role in inflammatory processes (Halliwell & Gutteridge 1999). Antioxidants scavenge reactive oxygen intermediates and protect the organism at the cellular and molecular level against oxidative damage, and may thus contribute to the prevention of diseases. Protective effects are attributed to several dietary antioxidants, including flavonoids, vitamin E, vitamin C and carotenoids (Sies & Stahl 1995; Rice-Evans 1999). It has been suggested that dietary supplements or other pharmaceutical preparations that are rich in specific antioxidants may improve the antioxidant status of the organism and contribute to the preventive effects of a healthy diet.

Turmeric (Curcuma longa L., Zingiberaceae) rhizome extracts are rich in the yellow-reddish pigment, curcumin (Figure 1), and other curcuminoids, derivatives of hydroxycinnamic acid. Curcumin has been shown to exhibit anticarcinogenic activity in animal models and possesses anti-inflammatory properties. The compound is a potent inhibitor of enzymes that generate reactive oxygen or nitrogen species, such as

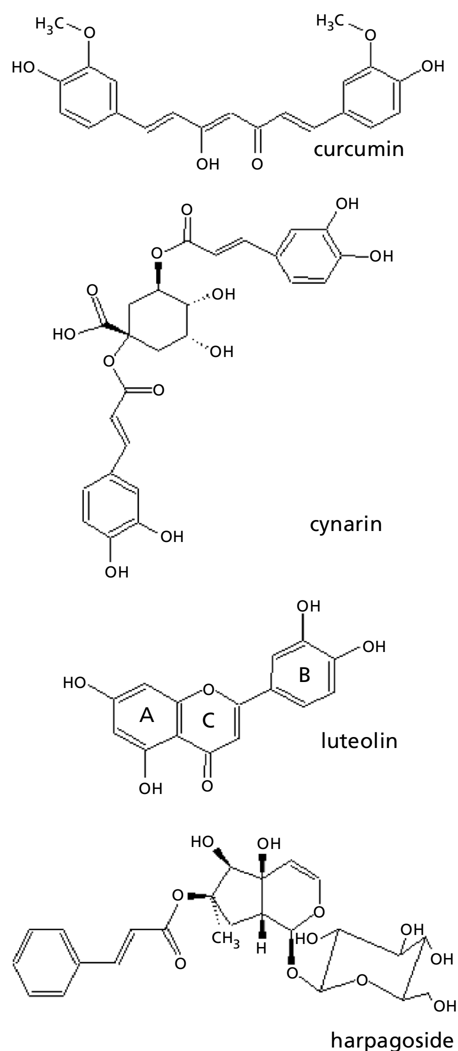

Figure 1 Selected compounds present in the test preparations.

lipoxygenase, cyclooxygenase, xanthine oxidase and nitric oxide synthase. Curcumin also inhibits protein kinase C and epidermal growth factor receptor tyrosine kinase (Lin et al 2000). At present, turmeric extract is mainly used in the treatment of disorders of liver and gallbladder owing to its choleretic effects.

Pharmacological properties of artichoke (Cynara scolymus L., Compositae) leaf extracts are attributed to caffeoylquinic acids, such as chlorogenic acid, and their derivatives, mainly cynarin, but also to the variety of glycosides of the flavone luteolin (Figure 1). It is also used to treat disorders of liver and gallbladder, as well as dyspepsia, atherosclerosis and diabetes (Wegener & Fintelmann 1999).

Extracts of devil's claw (Harpagophytum procumbens [Burch.] D.C. ex Meissn., Pedaliaceae) roots are used in the treatment of arthritis (Chantre et al 2000). Iridoid glycosides, among them harpagoside, harpagide and procumbide, are the most abundant compounds in the roots. Flavonoids such as luteolin (Figure 1) and kaempferol are also present in small amounts.

Garlic (Allium sativum L., Amaryllidaceae) has been used for medical purposes for more than 3500 years. It exhibits cardioprotective, lipid-lowering and antithrombotic effects (Borek 2001). Garlic has been reported to protect against stomach and colorectal cancers. The protective effect appears to be related to the presence of organosulfur compounds (Bianchini & Vainio 2001).

Salmon oil concentrate is a suitable source of polyunsaturated n-3 fatty acids, such as eicosapentaenoic or docosahexaenoic acids. Dietary intervention studies provide evidence that an increased intake of n-3 fatty acids correlates with a low incidence of coronary heart disease (Harris & Isley 2001).

In the present study, the antioxidant capacity of five common pharmaceutical preparations derived from turmeric, artichoke, devil's claw, garlic and salmon was studied.

Materials and Methods

Materials

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium peroxodisulfate (potassium persulfate), Folin-Ciocalteau's reagent, luteolin, chlorogenic acid, β-carotene and gallic acid were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Kaempferol and curcumin were purchased from Extrasynthese (Lyon, France). Harpagoside was from Phytochem (Ichenhausen, Germany). RRR-α-tocopherol was obtained from Cognis Deutschland (Düsseldorf, Germany).

The following preparations were purchased at local pharmacies: capsules of Curcu-Truw, containing ethanolic extract of turmeric rhizomes (dry drug to solvent ratio 13–25:1, 27% curcuminoids, batch no. 104025) and of Rheuma-Sern, containing aqueous extract of devil's claw roots (2:1, 2.60% harpagoside, batch no. 01220229) (both from Truw Arzneimittel, Germany); capsules of Hepar-SL forte, containing aqueous extract of artichoke leaves (4-6:1, batch no. 02011027; Sertürner Arzneimittel, Germany); Kwai N, coated tablets with fresh garlic extract (standardized to 1.0–1.4% alliin, batch no. 02011173; Lichtwer Pharma, Germany), and Ameu, soft capsules containing salmon oil concentrate with 35% n-3 fatty acids minimum (batch no. 02013059; Omega Pharma, Germany).

Sample preparation

Pure compounds

Stock solutions (2 mM) of pure compounds were prepared by dissolving luteolin, kaempferol, chlorogenic acid, curcumin and α-tocopherol in ethanol, β-carotene in dichloromethane, harpagoside in water, and Trolox in both water and ethanol.

Pharmaceutical preparations

The content weight of each capsule of turmeric, devil's claw, artichoke and salmon oil was calculated as the mean

difference in weight between the whole capsule and the capsule cover (n = 7). (i) Fractions A–C. Five whole garlic coated tablets and the contents of four to six capsules of turmeric, devil's claw or artichoke extract were crushed in a mortar until homogeneity of particle size. The products were treated with solvents of different lipophilicity to obtain fractions for testing. To 10–100 mg of powder, either 1 mL of water, methanol/water (70:30), or ethanol was added. After 24 h of incubation under agitation at 4 °C in the dark, all suspensions were centrifuged at 2570 g for 10 min and the supernatants collected. The pellets were washed with 0.5 mL solvent, left for 2 h at 4 °C in the dark and centrifuged. Supernatants from the same solvent were combined. The extracts were designated as fraction A (water), fraction B (methanol/water (70:30), and fraction C (ethanol). The clear supernatants and the solutions of the pure compounds were stored at −80 °C until use. Storage time was less than 7 days. (ii) Fraction D: extraction of highly lipophilic compounds. A highly lipophilic fraction (fraction D) was prepared as follows. Powder (1 g) was suspended in 50 mL acetone/water (75:25 v/v) for 2 h in the dark and vacuum-filtered through a Büchner funnel. The residue was extracted again until the filtrate was colourless. The filtrates were transferred to a decanting funnel; 150 mL of diethyl ether was added, and the funnel was shaken. Bidistilled water was added to separate the phases. The upper phase, which contained the lipophilic compounds, was washed several times with water. The ether solution was filtered through a solid bed of Na_2SO_4 and dried in a rotavapor. The residue was dissolved in acetone and named fraction D. Fraction D of salmon oil was a 200 mg mL^{-1} solution of the capsule contents in hexane.

Determination of total phenols

The amount of total phenols in the fractions was determined according to the Folin-Ciocalteau method (Singleton & Rossi 1965). The sample (0.1 mL) was diluted with 5.4 mL bidistilled water and mixed with 4 mL of 7.5% sodium carbonate and 0.5 mL of 2 N Folin-Ciocalteau's reagent. After 2 h incubation in the dark, absorbance was measured at 765 nm. Gallic acid was used as a standard.

Carotenoid analysis by high-performance liquid chromatography

The high-performance liquid chromatography system consisted of a Merck-Hitachi L-7100 pump connected with a Merck-Hitachi UV/vis detector set at 450 nm. Chromatographic separation was performed on a reversed phase column (Spherisorb C-18 ODS2; particle size = 5 μm; 250 mm × 4 mm). The eluent comprised a binary gradient at a constant flow of 1.5 mL min^{-1}. The initial composition of the eluent (acetone/water 75:25) was held for 5 min. Then, a linear gradient was applied for 5 min to yield a final composition of acetone/water of 95:5. This composition was held for 7 min. Finally, the column was washed for 3 min with acetone (Mínguez-Mosquera & Hornero-Méndez 1993). Carotenoids present in fraction D were identified by UV/vis spectroscopy and retention times on the column. For comparison, purified standards isolated from natural sources were used, including β-carotene, violaxanthin, zeaxanthin, lycopene and β-cryptoxanthin. For quantification, the external standard method was applied.

Determination of antioxidant capacity using the Trolox equivalent antioxidant capacity assay

The antioxidant capacity of the pure compounds and the different samples derived from the commercial preparations was measured using the Trolox equivalent antioxidant capacity (TEAC) assay (Re et al 2000). The assay is based on the ability of compounds to scavenge the $ABTS^{\bullet+}$ radical cation; data are given as equivalents of the reference compound, Trolox. The $ABTS^{\bullet+}$ radical was generated by adding 5 mL of a 4.9-mM potassium persulfate solution to 5 mL of a 14-mmol L^{-1} ABTS solution in water. The reaction was complete after 6 h; no further increase in the absorbance at 734 nm was detected after that time. The $ABTS^{\bullet+}$ solution was diluted to obtain an absorbance of 0.70 absorbance units (734 nm) and pre-incubated at 30 °C. When the samples were dissolved in ethanol, hexane or dichloromethane, the $ABTS^{\bullet+}$ solution was diluted in ethanol. For samples dissolved in water, acetone, or methanol/water (70:30), dilution was with water. The sample solution (10 μL) was added to 1 mL of the $ABTS^{\bullet+}$ solution. The mixtures were vortexed briefly and the absorbance at 734 nm was measured 3, 6 and 9 min after addition (Beckman DU 530 spectrophotometer; Beckman Instruments, Fullerton, USA). Solvent controls were measured in each series. Pure compounds were tested in triplicate at four concentrations. Five replicates of every fraction were prepared and at least four dilutions of each were tested. The loss of absorbance (%) at 734 nm determined after 9 min was plotted against the concentration of the sample. This time point was selected because some reactions were not completed before then. The slope calculated for the test compound was divided by the slope obtained for Trolox (reference compound). Thus, the antioxidant activity is expressed in Trolox equivalents (TE).

Statistical analysis

Statistical analysis was performed using the Statistica 5.5 (1999) software package for Windows (Statsoft, Inc., Tulsa, OK, USA). TEAC and total phenols data obtained for the four fractions of each preparation (except for salmon oil) were analysed using the Friedman's test. Individual values were compared using the Mann–Whitney U-test. Multiple regression analysis was performed between TEAC and total phenols results. The effect of concentration on $ABTS^{\bullet+}$reduction was assessed using the Kruskal–Wallis test. A significant difference was considered at $P < 0.05$.

Results

TEAC values determined for the pure compounds are shown in Table 1. In the ABTS assay, curcumin, luteolin, kaempferol, chlorogenic acid and β-carotene exhibited an antioxidant activity superior to Trolox (1 per definition) as expressed in TE. α-Tocopherol had about the same activity, whereas harpagoside showed only 4% of the Trolox activity. The data obtained for the different fractions of the preparations are shown in Figure 2. TEAC is given as μmol TE (g dry matter)$^{-1}$ (capsule contents or whole coated tablets). A linear relationship between concentration and response was found for all compounds and fractions, except for curcumin and fractions B, C and D of the turmeric extract preparation, where a biphasic response was observed (Figure 3). Here, fractions C and D provided 100% protection at 0.15 and 0.5 mg dry matter mL^{-1}, respectively, and fractions A and B were less efficient. The linear response range of curcumin was from 0 to ~4 μmol L^{-1} (data not shown); the TEAC values for curcumin and turmeric fractions were calculated using the slope in the linear range. A significant concentration-dependent effect of the turmeric preparation on $ABTS^{\bullet +}$ reduction was found ($P < 0.05$).

Curcumin was the major polyphenol in the ethanolic extract of turmeric. The UV/vis spectrum obtained from fraction C of the turmeric preparation was similar to that of curcumin in ethanol, showing a maximum at 424 nm. On the basis of the content of curcuminoids provided by the manufacturer, and the TEAC value determined for curcumin, the antioxidant activity of fraction C (1257 ± 82 μmol TE g^{-1}) was mainly attributed to the presence of curcumin (~90%). In fraction D, where carotenoids are most soluble, only β-carotene was found corresponding to 0.69 μg (g dry matter)$^{-1}$, implying that carotenoids did not account for the rest of the antioxidant activity.

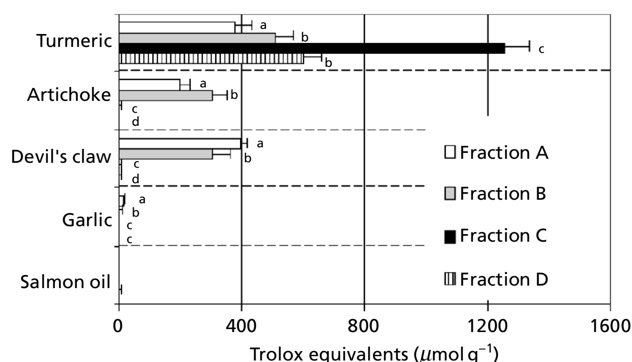

Figure 2 Trolox equivalent antioxidant capacity (TEAC) of preparations containing extracts of turmeric, artichoke, devil's claw and garlic or salmon oil. Fractions were obtained by treating the contents with solvents of different polarity: water (fraction A), methanol/water (70:30 v/v) (fraction B), ethanol (fraction C) and acetone/water (75:25 v/v) followed by diethyl ether (fraction D). Fraction D of salmon oil consisted of a solution of the entire extract in hexane. Data are mean ± s. d., n = 5 (each fraction was tested at least at four concentrations). [a,b,c,d]Different letters indicate significant differences between two treatments; $P < 0.05$.

The highest TEAC for the artichoke leaf product was found in fraction B, corresponding to 302 ± 46 μmol TE g^{-1} (Figure 2). Fraction A showed activity that was somewhat less. Very little antioxidant activity was found in fractions C and D.

The antioxidant activities measured as TEAC for two abundant compounds in artichoke leaves, luteolin and chlorogenic acid, were 2.18 ± 0.10 and 1.20 ± 0.08 μmol TE μmol^{-1}, respectively (Table 1). No carotenoids were found in fraction D.

Devil's claw extract is particularly rich in water-soluble antioxidants, since the major TEAC was found in fractions A and B (Figure 2). Harpagoside is considered as an

Table 1 Trolox equivalent antioxidant capacity (TEAC) of selected compounds.

Test compound	TEAC (μmol TE μmol^{-1})
Curcumin	1.92 ± 0.09
Luteolin	2.18 ± 0.10
Kaempferol	1.39 ± 0.09
Chlorogenic acid	1.20 ± 0.08
Harpagoside	0.04 ± 0.02
β-Carotene	2.34 ± 0.07
RRR-α-Tocopherol	0.97 ± 0.04

The loss of absorbance at 734 nm (%) as a result of ABTS radical reduction was plotted against the concentration of the test compound. The slopes obtained were divided by the slope corresponding to Trolox (reference compound). Thus, the antioxidant activity is expressed in Trolox equivalents (TE). Results are given as mean ± s.d., n = 3 (each compound was measured in triplicate at four concentrations).

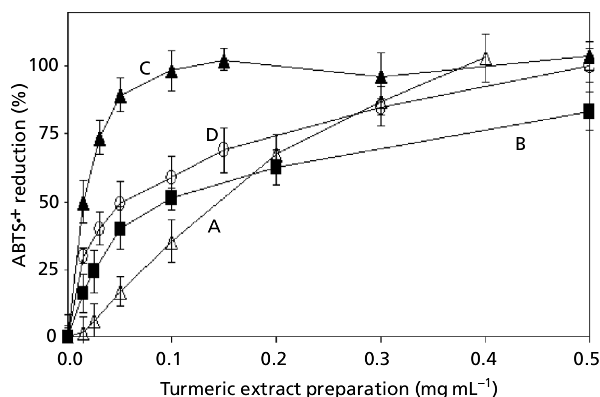

Figure 3 Reduction of the 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation in the presence of fractions A–D obtained from the turmeric preparation. Concentration is expressed as mg dry matter equivalents mL^{-1}. Data are mean ± s.d., n = 3–5. The effect of concentration on $ABTS^{\bullet +}$ reduction was significant within all fractions as analysed by the Kruskal–Wallis test.

active compound in devil's claw, but very poor antioxidant activity was found in the TEAC assay (Table 1). In fractions C and D, very low activity was detected. Traces of β-carotene ($2.34\,\mu g\,g^{-1}$) and β-cryptoxanthin ($< 0.1\,\mu g\,g^{-1}$) were found in fraction D. The antioxidant activity of kaempferol, a flavonoid found in devil's claw roots, was $1.39 \pm 0.09\,\mu$mol TE μmol^{-1}.

Fractions A and B of the garlic coated tablets exerted some antioxidant activity in the TEAC assay, slightly higher for the water-soluble fractions, whereas fractions C and D showed almost no effect. No carotenoids were found in fraction D.

Fraction D of the salmon oil, corresponding to the entire oil dissolved in hexane and the non-saponifiable fraction containing the added vitamin E, showed very similar antioxidant activity (approx. $4\,\mu$mol TE g^{-1}). The α-tocopherol concentration was $1.85\,mg\,g^{-1}$ of oil as determined photometrically. In fraction D, no carotenoids were found.

The concentration of Folin-active compounds was particularly high in the turmeric fractions, followed by devil's claw and artichoke. Good linear regression coefficients were achieved between TEAC values and total phenols content in all test preparations.

Discussion

The TEAC values of the pure compounds (Table 1) tested in the present study were in accordance with published data (Rice-Evans & Miller 1998; Re et al 2000), except for curcumin (1.92 TE), which was lower than described previously with 3.09 TE (Venkatesan & Rao 2000). This might be owing to differences in the mode of calculating TE. Here, we used a comparison of the slopes, whereas IC50 values were used for the calculation by Venkatesan & Rao (2000).

Curcuminoids are polyphenols and act as classical chain-breaking antioxidants like vitamin E. Their antioxidant activity decreases in hydrogen bond forming media; synthetic non-phenolic curcuminoids are mostly inactive (Barclay et al 2000). The presence of an electron donor at the ortho position to the hydroxyl group, such as the methoxy group in curcumin, exerts an inductive effect that facilitates the transfer of the hydrogen atom and disturbs the formation of hydrogen bonds.

The biphasic responses obtained for curcumin and the turmeric fractions B, C and D (Figure 3) are explained as a result of curcumin radicals being formed and coupled in the reaction mixture to yield insoluble dimeres and polymeres that precipitate (Masuda et al 1999). Such reactions were favoured in the present system at curcumin concentrations above $4\,\mu mol\,L^{-1}$. A range of stable tricyclic compounds resulting from coupling reactions between curcumin radicals and the peroxyl radicals derived from polyunsaturated fatty acids has been identified (Masuda et al 2001).

The contribution of β-carotene, the single carotenoid detected, to the antioxidant capacity was less than 0.1% in relation to the absolute amount. Thus, further antioxidant compounds must be present, since the water-soluble fraction A shows high activity. Phenolic decomposition products of curcumin, such as ferulic acid, vanillin or vanillic acid (Masuda et al 1999) may account for this effect, since this fraction was rich in phenols (Table 2), but curcumin is insoluble in water. Interestingly, the TEAC value for ferulic acid, which can be considered as a curcumin monomere, has been shown to be about half the value for curcumin (Pannala et al 2001).

The major antioxidant capacity of the artichoke leaf preparation was found in fractions A and B, coinciding with the presence of phenols. Most flavonoid glucosides as well as hydroxycinnamates are soluble in these solvents (Macheix & Fleuriet 1998). Artichoke leaves are particularly rich in caffeic acid and esters with quinic acid, such as chlorogenic acid and the diester cynarin (Figure 1), which is almost exclusively found in artichokes. Water was the extraction solvent used for the preparation of the commercial product, therefore the low antioxidant properties found in the more lipophilic fractions C and D are reasonable.

Luteolin, caffeic acid and cynarin all share a catechol ring, accounting for their better reduction potential in comparison with hindered or single phenols. The oxidation of catechol-containing flavonoids likely consists of a two-electron donation process, yielding first semiquinones and then ortho-quinones (Jovanovic et al 1998; Pannala

Table 2 Total phenol content on treatment with solvents of different polarity and relation to the antioxidant activity.

Preparation	n	Total phenols (mg gallic acid equivalents (g dry matter)$^{-1}$)				R (TEAC/total phenols)
		Fraction A	Fraction B	Fraction C	Fraction D	
Turmeric	5	30.1 ± 2.8^{a}	46.0 ± 4.1^{b}	124.7 ± 6.0^{c}	46.2 ± 4.6^{d}	0.983 ($P < 0.01$)
Artichoke	5	14.8 ± 3.1^{a}	25.5 ± 6.9^{b}	1.0 ± 0.5^{c}	0.0 ± 0.0^{d}	0.973 ($P < 0.01$)
Devil's claw	5	37.4 ± 4.5^{a}	29.9 ± 7.1^{a}	0.7 ± 0.2^{b}	0.3 ± 0.1^{c}	0.986 ($P < 0.01$)
Garlic	5	0.8 ± 0.4^{a}	0.9 ± 0.3^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.688 ($P < 0.01$)
Salmon	5	—	—	—	0.5 ± 0.1	—

Values are mean ± s.d. [a,b,c,d]Different letters indicate significant differences between two treatments within one preparation; $P < 0.05$. R represents the linear regression coefficient between Trolox equivalent antioxidant capacity (TEAC) and total phenols values for each preparation.

et al 2001), whereas hindered or single phenols react via formation of phenoxyl radicals.

Results for the devil's claw preparation are consistent with the fact that water-extractable substances account for about 70% of the dry weight of the roots. The potential antioxidant activity of harpagoside, owing to the presence of hydroxyl groups in the bicyclic ring, was not confirmed (Table 1). A contribution of other iridoids, such as harpagide or procumbide, to the antioxidant activity is excluded because of their high structural similarity with harpagoside. According to linear regression data, phenols significantly contribute to the antioxidant activity. Major phenols in devil's claw roots are glycated forms of flavonoids like kaempferol or luteolin. Kaempferol is a monophenolic flavonol and it exhibits a lower antioxidant activity than luteolin (Table 1).

The garlic tablets showed low antioxidant activity in all fractions. There are more water-soluble than lipid-soluble antioxidants present in garlic extract. TEAC and total phenols correlated among treatments, but other compounds present in garlic extract are also Folin-active, particularly containing sulfhydryl and disulfide groups.

Results for the salmon oil revealed that all the antioxidant activity found in the oil in solution was related to the α-tocopherol added to the product for stabilization (Figure 2).

It should be noted that mixtures of antioxidants, as present in plant extracts, may exert synergistic effects and can be of superior activity to single compounds (Pedrielli & Skibsted 2002). On the other hand, the results obtained for each fraction of each preparation are not additive, as some antioxidants may dissolve in more than one medium. Since all experiments were performed in an in-vitro system, results are irrespective of absorption of the nutrients in the digestive tract and, by extension, of their activity in-vivo and metabolism.

Conclusion

Extracts of turmeric rhizome exhibit pronounced antioxidant activity, as estimated with the TEAC assay, which is attributable to the presence of curcumin. The antioxidant activity shown by devil's claw extract is not attributed to harpagoside. In general, the antioxidant capacity of plant extracts can be ascribed mainly the presence of phenols. Antioxidant properties of natural plant products may contribute to their therapeutic efficacy and provide additional health effects.

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