Comparative study of two natural antioxidants, curcumin and *Curcuma longa* extract

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Summary

The antioxidant capacities of curcumin and turmeric extract were determined and compared. Turmeric extract showed higher values of antioxidant power normalized to curcumin content (31.9%) in a majority of antioxidant assays than the curcumin standard. The particular half-maximal inhibitory concentration (IC_{50}) values for curcumin were 2.34 $\mu g \cdot ml^{-1}$ in the 1,1'-diphenyl-2-picrylhydrazyl (DPPH) test, and 0.21 $mg \cdot ml^{-1}$ in the liposomal peroxidation assay in the extract, compared to IC_{50} = 3.33 $\mu g \cdot ml^{-1}$ and 0.343 $mg \cdot ml^{-1}$ in the curcumin standard, respectively. Conversely, a significantly lower IC_{50} of curcumin (0.020 $mg \cdot ml^{-1}$) for 12-lipoxygenase (LOX) inhibition was obtained than that of *C. longa* extract (2.95 $mg \cdot ml^{-1}$). The reactivity of the samples tested in the particular assays correlated well with the redox potentials of the model oxidants. In the liposomal system, the antioxidant activity may be influenced by the distribution processes at the phase interface. In conclusion, our results suggest that curcumin and its analogues may be decisive for the free radical-scavenging capacity of the turmeric extract.

Keywords

Curcuma longa L.; curcumin; anti-inflammatory agents; antioxidant agents; lipoxygenase

Curcumin (diferuloylmethane), the major bioactive compound of the turmeric used in Indian cuisine as a component of curry powder and as a colouring agent, belongs to the most imperative phenolic compounds, which undergo systematic research. Turmeric is a ground powder obtained from the root of *Curcuma longa* L. (*Zingiberaceae*) [1], containing, besides curcumin, also its analogues with different substituents on the phenyl rings. Curcuminoids have been shown to exert beneficial effects in the inflammatory processes that play a major role in most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases [2, 3]. In this regard, these compounds have been shown to regulate numerous transcription factors, cytokines, protein kinases, adhesion molecules, enzymes and also the redox status. Additionally, a number of studies have provided evidence of chemosensitizing, radiosensitizing, wound healing activities, antimicrobial, antiviral and antifungal properties of curcumin and its derivatives [4]. Research also supports the opinion of a wide-ranging health benefit of curcumin-related compounds in the treatment of multiple diseases that consists in its capacity to reduce the oxidative stress [5]. Both antioxidant and prooxidant activity have been reported for curcumin and its analogues [6, 7]. A broad interest in the activities of curcumin prompted us to revise its antioxidant activity using several methods, in comparison to the effect of the crude turmeric extract.

MATERIALS AND METHODS

Plant material

The commercially available standard compound, curcumin from *Curcuma longa* root, ob-

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tained from Sigma (St. Luis, Missouri, USA), had purity of 70% (determined by HPLC; desmethoxycurcumin and bisdesmethoxycurcumin formed the remaining 30%). Turmeric, the dried powdered dry rhizome of *C. longa* plant was obtained from Vido (Bratislava, Slovakia) in September 2006.

Chemicals

2,2'-Azinobis-3-ethylbenzthiazino-6-sulfonic acid (ABTS), sodium nitroprusside, ascorbic acid and 2,2'-azo-bis-(2-aminidopropane)-hydrochloride (AAPH) were purchased from Fluka Chemie (Buchs, Germany). DPPH (1,1'-diphenyl-2-picrylhydrazyl) radical, linoleic acid (99%), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (C18:1, *cis*-9, DOPC, 99% grade) and Trolox were purchased from Sigma. 2,4,6-tripyridyl-*s*-triazine (TPTZ) was from Merck (Darmstadt, Germany). All other chemicals used were of the highest available analytical grade.

Extraction and analysis of curcuminoid composition of *Curcuma longa*

Dried finely powdered rhizome (500 g) from *Curcuma longa* L. was macerated twice with 70% ethanol at a ratio of 15 : 100 (m/v) at room temperature for two days and then filtered. Following concentration and freeze-drying, the analysis of curcuminoid composition of the crude extract was carried out by reversed-phase HPLC (Ecom, Prague, Czech Republic) on Reprosil 100 C18 5 μm, 250 mm × 4 mm (Watrex, Prague, Czech Republic) using a mixture of methanol - isopropanol - water - acetic acid (20 : 27 : 48 : 5) as the mobile phase and UV-VIS detection (LCD 2082, Ecom), as described earlier [8]. Total concentration of curcuminoids in the turmeric extract was 60.6% (in a ratio of 3 : 1 : 1.7 for curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively) and was identified on the basis of the retention time by using curcumin from Sigma as a standard.

DPPH, ABTS and NO radical-scavenging activity

DPPH and ABTS assays were performed according to Wong et al. [9] and Re et al. [10], respecvtively. The absorbance decreases after 30 min and 6 min of the solution of DPPH and $ABTS^{+}\bullet$ in ethanol at 518 nm and 734 nm, respectively, were recorded following the addition of the tested samples. The NO assay using sodium nitroprusside was carried out as described earlier [11]. At the end of the incubation of the sample with sodium nitroprusside, Griess reagent was added to the reaction mixtures at a 1 : 1 volume ratio. The absorbance of the chromophore formed was followed at 540 nm, the readings being made in triplicate. The concentration of the compound causing the absorbance decrease by 50% compared to the sample without antioxidant (IC_{50}) was taken as the parameter of the free radical-scavenging potency.

Inhibition of non-enzymatic lipid peroxidation

Peroxidation of the liposomal membrane was triggered by thermal decomposition of AAPH. Liposomes were prepared and lipid extraction was carried out using previously described methods [12]. Suspension of unilamellar liposomes (1 mM DOPC) was prepared by dissolving DOPC (15.7 mg) in chloroform (5 ml) in a round-bottom flask. The solvent was subsequently removed under nitrogen gas and the resulting thin film on the walls was dispersed in phosphate buffer (20 ml, 20 mM, pH 7.4) by vigorous stirring followed by sonification. The liposomes (final concentration of 0.8 mM DOPC) were incubated in the presence of different concentrations of the antioxidants tested and of the initiator AAPH (final concentration of 10 $mmol \cdot l^{-1}$) at 50 °C for 80 min. The incubation mixtures were extracted with 2 ml portions of an ice-cold mixture $CHCl_3$/MeOH (2 : 1, v/v), containing BHT (0.05%). The lipid hydroperoxide content was determined in the extracts by the thiocyanate method. The amount of $Fe(SCN)_3$ formed was followed spectrophotometrically at 500 nm. The concentrations of the compounds causing 50% absorbance decrease of the liposomal extracts compared to the sample without the antioxidant (IC_{50}) were evaluated. In order to obtain a linear dependence, concentration data in the DOPC-peroxidation assay were converted to logarithms.

Inhibition of lipid peroxidation catalysed by 12-lipoxygenase (LOX)

The assay was carried out using the isolated cytosolic fraction from rat lung as described earlier [13]. The enzymatic reaction was monitored spectrophotometrically at 234 nm for 5 min. The concentrations of the compounds tested causing 50% absorbance decrease of the liposomal extracts compared to the sample without the antioxidant were taken as IC_{50} values.

Ferric-reducing antioxidant power (FRAP)

The ferric-reducing power assay was performed using the FRAP assay as described by Benzie and Strain [14]. The conversion of Fe^{3+}-TPTZ complex to the ferrous form was monitored at 593 nm for 6 min at room temperature. Absorbance values were converted to μmol Trolox equivalent per μg of the substance tested, based on a calibration curve.

Tab. 1. Free radical-reducing capacities of the curcumin standard and *Curcuma longa* extract.

	DPPH test	ABTS test	NO scavenging assay	DOPC peroxi-dation	LOX catalysed peroxidation	FRAP
	IC_{50} [$\mu g \cdot ml^{-1}$]			IC_{50} [$mg \cdot ml^{-1}$]		TE [$\mu mol \cdot \mu g^{-1}$]
curcumin [a]	3.33 ± 0.02	0.77 ± 0.02	10.5 ± 0.2	0.343 ± 0.02	0.020 ± 0.31	4.29 ± 0.31
C.longa extract [a]	2.34 ± 0.11	0.49 ± 0.06	12.4 ± 1.3	0.21 ± 0.03	2.95 ± 0.60	5.33 ± 0.60
curcumin [b]	4.76 ± 0.02	1.10 ± 0.02	14.5 ± 0.2	0.49 ± 0.02	0.029 ± 0.31	3 ± 0.2.10-3
C.longa extract [b]	4.45 ± 0.11	0.921 ± 0.06	23.63 ± 1.3	0.40 ± 0.03	5.60 ± 0.6	2.8 ± 0.4.10-3

Values are expressed as mean ± SD (n = 3). TE – Trolox equivalent.
a – results are normalized to curcumin content in the tested samples, *b* – results are normalized to total curcuminoid content in the tested samples.

RESULTS AND DISCUSSION

Since the analysis of *Curcuma longa* composition showed the content of 31.9% of curcumin and the purity of the curcumin standard was 70%, we normalized the results of antioxidant capacity to the curcumin content. The results expressed in this way showed the increased antioxidant activity of curcumin in *C. longa* extract in comparison to that in the curcumin standard in DPPH- and ABTS-scavenging assays, peroxidation of DOPC liposomes and FRAP assay (Tab. 1, Fig. 1A, 2). These results emphasize the role of synergistic effects of other constituents of *C. longa* extract on the antioxidant activity of curcumin. This is in concordance with Liu et al. [15], who documented the synergistic effect influencing DPPH radical scavenging in combination with natural antioxidants. The mechanism of the synergistic effects of various nucleophiles on the radical scavenging ability of plant polyphenols containing a 3,4-dihydroxy substructure has been proposed [16].

However, since the portion of 30% in the curcumin standard comprises other curcuminoids possessing comparable radical scavenging efficacies [17], their contribution to the antioxidant capacity cannot be neglected. Re-calculation of the results respecting the total curcuminoid content gave a good fit between the results of antioxidant capacity of the curcuminoid standard and the

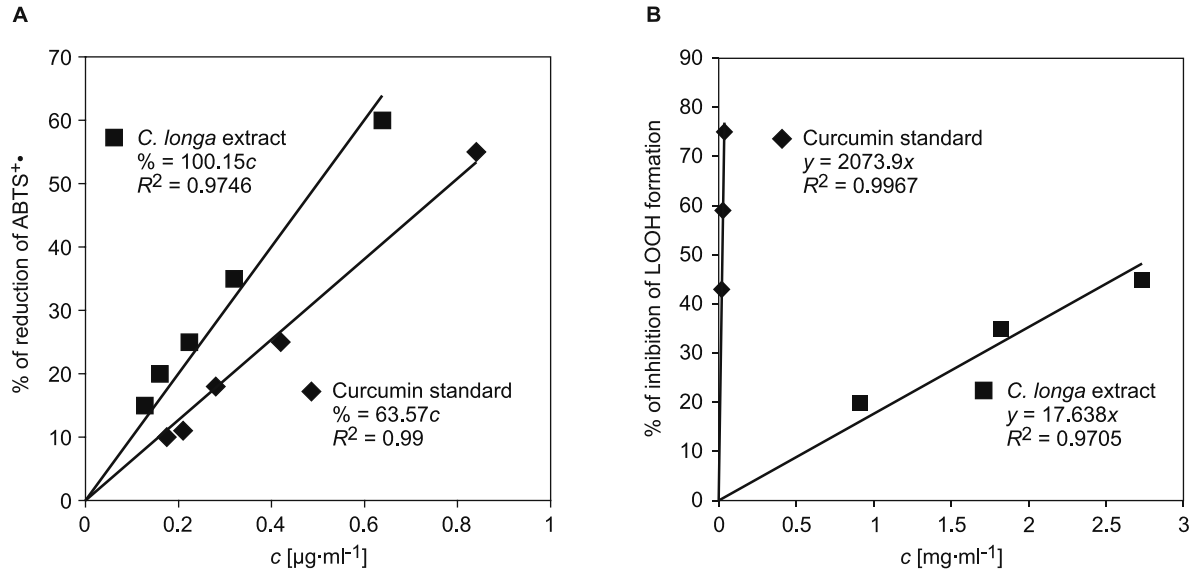

Fig. 1. Free radical-scavenging capacities of curcumin and *Curcuma longa* extract expressed as a function of the concentration of curcumin in the samples tested in ABTS test (A) and LOX-catalysed linoleic acid hydroperoxide (LOOH) formation (B).
The IC_{50} values were calculated from the respective linear regression equations.

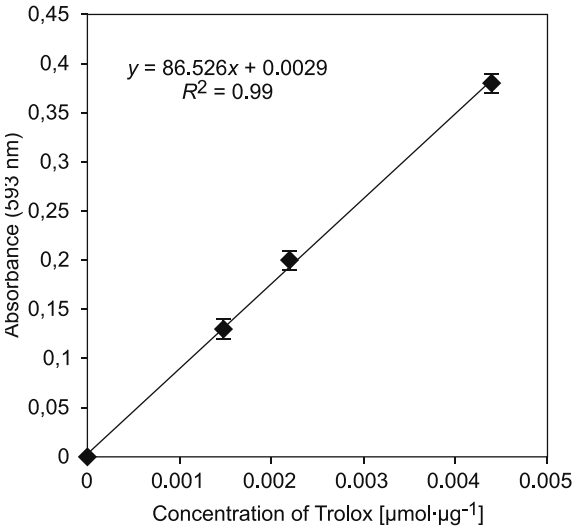

Fig. 2. Calibration curves of the reduction of ferricyanide complexes by ascorbic acid.

crude extract, which comprised 60.6% of the curcuminoid content (Tab. 1).

Although we found a similar activity profile in the range of assays used, the reactivity of the extract and of curcumin significantly differed between individual model oxidant systems (Tab. 1). The decreasing reactivity with radicals from $ABTS^{+\cdot}$ towards $DPPH^{\cdot}$ and $NO^{\cdot}$ radical (reflected by the concentration causing the half-maximum absorbance decrease in the colorimetric assays; Tab. 1, Fig. 1A) correlated well with their redox potentials, with approximate values versus normal hydrogen electrode ($ABTS^{\cdot+}$/ABTS of 0.68 V, DPPH/$DPPH^{-}$ of 0.43 V [18], NO/NO^{-} in a range from 0.4 V to –1 V [19]). However, although the redox potential of AAPH-derived radical species (ROO•, RO•, OH•) ranging between 1.0–2.3 V [18], predisposed them for a higher reactivity with the antioxidants tested, the IC_{50} values in the peroxidation of DOPC liposomes showed higher values than those in the mentioned assays. This disproportion may arise from the presence of the lipid-water interface in the assay system associated with distribution processes of the compounds, which may limit their availability for the reaction with free radicals. Correspondingly to the reported data [20], curcumin or its analogues in the turmeric extract, reported as highly lipophilic compounds [21], may be limited in their access to chain-initiating peroxyl radicals due to their deeper location within the liposomal membrane.

Regardless of the method of evaluation of its activity, the curcumin standard showed a higher antioxidant activity than the extract in the model of enzymatic lipid peroxidation, which cannot be explained only by the reduction of non-heme bound iron or direct scavenging of lipidoxy- or lipidperoxy- radicals (Fig. 1B). Curcumin may also interact with LOX catalytic center through chelating of iron (Fe^{2+}) [22] and non-competitive type of LOX inhibition cannot be excluded [23].

In conclusion, the present study provides some useful insights into the antioxidant potency of *C. longa* extract and its major constituent, curcumin. Our results suggest that content of curcumin or its analogues may be decisive for free radical-scavenging capacity of the turmeric extract.

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