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Antioxidant properties of curcuminoids isolated from *Curcuma longa* L.

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Abstract: The evaluation of antioxidant potential of food has received much attention in recent years. Antioxidant compounds can scavenge free radicals and thereby can protect the human body from free radicals. This study was focused on the isolation of curcuminoids from the dried turmeric rhizome, and studying their antioxidant activity. The presence of curcuminoids was identified in turmeric sample by Nuclear Magnetic Resonance (NMR) analysis. Since neutral curcumin is known to be poorly soluble, the synthesis of curcumin-cyclodextrin and curcumin-phospholipid complexes was also performed. The antioxidant activity of isolated curcuminoids was assessed by two methods (ABTS and FRAP assay) and their scavenging activities were compared with those of prepared complexes. The ability to reduce ABTS radical cation decreased as follows: quercetin > trolox > curcuminoids > curcumin-cyclodextrin complex > curcumin-phospholipid complex. The reducing potential of tested samples in descending order was quercetin > trolox > curcumin-cyclodextrin complex > curcuminoids > curcumin-phospholipid complex.

Keywords: turmeric rhizome, curcuminoids, NMR analysis, ABTS test, FRAP assay

Introduction

Curcumin (turmeric) is a natural yellow orange dye derived from the rhizome of the plant *Curcuma longa* Linn., belonging to Zingiberaceae family and usually used as a spice (Gounder and Lingmallu, 2012). It is insoluble in water and ether but is soluble in ethanol, dimethylsulfoxide and other organic solvents. Curcuminoids consist of three major components including curcumin (77 %), demethoxycurcumin (17 %) and bisdemethoxycurcumin (3 %) (Aggarwal et al., 2003).

The major secondary metabolites of turmeric, the curcuminoid pigment(s) and volatile oils, have been shown to be largely responsible for the pharmacological activities of turmeric powder, extracts and oleoresins (Gounder and Lingmallu, 2012). Their scavenging activities against a variety of reactive oxygen species including superoxide anion radicals and nitrogen dioxide radicals are predominant (Yadav et al., 2009). They are also inhibitors of lipid peroxidation in different animal models (Maheshwari and Singh, 2006). On the other hand, curcuminoids have also exhibited pro-oxidant effects in the presence of Cu^{2+}. According to Ahsan et al. (1999), both antioxidant and pro-oxidant effects of curcuminoids are determined by the same structural pattern. Thus, an antioxidant in one system is not an antioxidant in all systems.

The oxygen radical scavenging activity of curcumin has also been implicated in its anti-inflammatory effects. Curcumin inhibits metabolism of arachidonic acid, cyclooxygenase (COX) and lipoxygenase (LOX) activities and releasing of steroidal hormones. It reduces pro-inflammatory leukotriene synthesis via inhibition of LOX enzyme. Curcumin is also a potent inhibitor of pro-inflammatory cytokines (Kohli et al., 2005).

The objective of the present study is to evaluate the antioxidant activity of curcuminoids isolated from turmeric. The presence of curcumin in obtained curcuminoids was also confirmed.

Material and methods

Sample

Turmeric rhizome powder BIO (*Curcuma longa* L.) was bought in local shops. Best before: 07. 2016; Lot number: SO14081315.

Isolation of curcuminoids from turmeric rhizome

Dried turmeric rhizome powder (200 g) was percolated using 600 ml of hexane at room temperature for 24 h for removal of volatile oil. After percolation, this percolate was filtered by Büchner funnel

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and then it was evaporated to dryness in a rotary evaporator at temperature lower than 40 °C. Curcuminoids from dried sediment were extracted using 600 ml of 95 % ethanol at room temperature for 72 h (two times repeated). Then, extract of curcuminoids was filtered by means of Büchner funnel, and evaporated to dryness in a rotary evaporator at temperature lower than 50 °C. The residue was dissolved in ethyl acetate and curcumoinds were precipitated from ethyl acetate solution by hexane. Obtained curcumin crystals were dissolved in methanol (antioxidant activity) and deuterated methanol (NMR analysis), respectively.

Analyses of turmeric sample using NMR spectroscopy
Qualitative analysis of curcuminoids presented in obtained turmeric extract was measured by nuclear magnetic resonance (NMR) according to Kaliňák et al. (2014). Briefly, ^{1}H-NMR spectra were run on a 600 MHz NMR spectrometer (Agilent) equipped with indirect detection probe. Deuterated methanol was used as solvent. The resulting ^{1}H-NMR spectra were processed using the MESTRENOVA program.

Preparation of curcumin complexes
Obtained curcuminoids were used for the preparation of complexes with cyclodextrin and phospholipid, respectively.

Curcumin-cyclodextrin complex by kneading method
Curcumin–cyclodextrin complex was prepared by kneading method according to Yadav et al. (2009). Both compounds were mixed in a mortar in the proportion of 1:1 for 1 h with small quantity of ethylalcohol. The paste was dried in a hot air oven at a temperature of 45 °C. Dried complex was pulverised into fine powder.

Curcumin-phospholipid complex
The curcumin-phospholipid complex was prepared at 1:4 ratio of curcumin and phospholipid (hydrogenated soy phosphatidyl choline) respectively. The mixture of curcumin, phospholipid, and ethyl alcohol was refluxed for 1 h with constant stirring on magnetic stirrer. The resultant clear solution was evaporated in a rotary evaporator to obtain curcumin-phospholipid complex.

Determination of antioxidant potentials
Radical scavenging potential of turmeric sample by ABTS radical cation assay
The free radical scavenging activity of curcuminoids was determined using ABTS radical cation decolourisation assay (Arts et al., 2004). Briefly, 0.05 ml of methanolic curcumin sample with different concentrations was added to 2 ml of ABTS reagent. The samples were kept at room temperature in the dark and after 10 min the absorbance at 730 nm was measured. The antioxidant activity of curcuminoids was expressed by IC_{50} value (mg/ml).

Ferric-reducing power (FRAP assay)
This assay (Niemeyer and Metzler, 2003) measures the ability of the antioxidants to reduce ferric-tripiridyltriazine (Fe^{3+}-TPTZ) to a ferrous form (Fe^{2+}). The main products of this reaction are the ferro- and ferric-iron complexes with TPTZ which absorb light at 595 nm. The antioxidant effect was also estimated as in ABTS test. The results represent the mean of two experiments.

Statistical analysis
Data are means ± standard deviation (SD) of two measurements.

Results and discussion

Analyses of turmeric sample using NMR spectroscopy
Determination of main constituent of the studied extract was done by NMR analysis according to Kaliňák et al. (2014) ^{1}H-NMR standard spectra were measured and compared with the reference spectra published by Payton et al. (2007) that confirmed the presence of curcumin (Fig. 1) in the turmeric extract (Fig. 2).

Antioxidant potentials
Curcumin is a phenolic antioxidant and its antioxidant activity is mainly due to the phenolic hydroxyl group (Tapal and Tiku, 2012). However, its

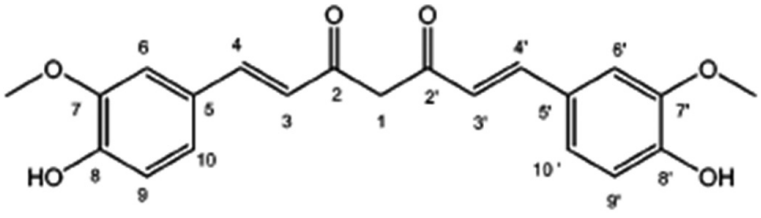


Fig. 1. Chemical structure of curcumin (Payton et al., 2007).

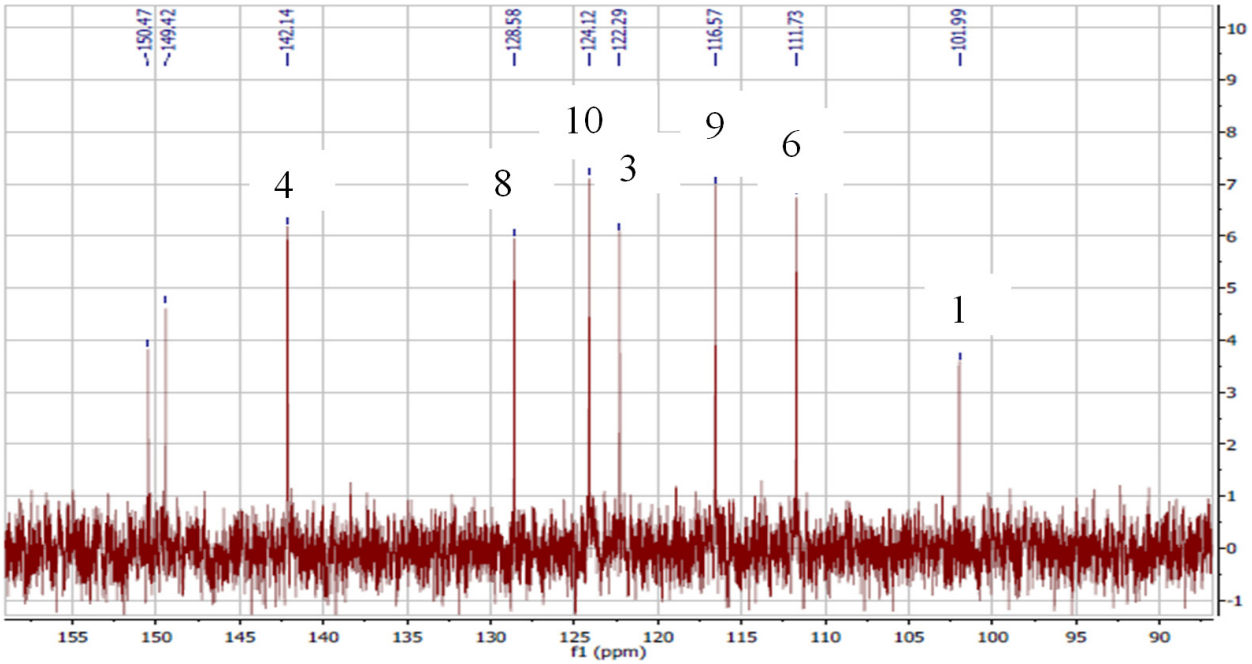


Fig. 2. Section of ^{1}H-NMR spectra of the turmeric extract. Numbers correspond to ^{1}H positions as presented in the Fig. 1.

extremely low aqueous solubility is a limiting factor in the clinical application of curcumin (Siviero et al., 2015). Most of the recent published papers are focused on the strategies to enhance the curcumin bioavailability. One of these strategies is the preparation of curcumin complexes with natural compounds including piperine, or quercetin. For this reason, we tried to prepare the curcumin-cyclodextrin and curcumin-phospholipid complexes to improve the lipophilic properties of curcuminoids. Then, antioxidant activities of curcumins complexes were estimated by ABTS and FRAP assay and compared to the antioxidant activity of curcumin.

ABTS radical scavenging method is based on the reduction of pre-formed radical cation ABTS•$^{+}$ by the addition of antioxidants (Gounder and Lingmallu, 2012). The extent of inhibition of ABTS•$^{+}$, expressed as the percentage radical inhibition, was plotted as a function of turmeric sample concentration and the result was expressed as IC_{50} (µg/ml) value.

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and lipophilic central cavity in which liphophilic drug is included to form hydrophilic drug/cyclodextrin complexes. The solubilizing effect of lipophilic drug is based on the

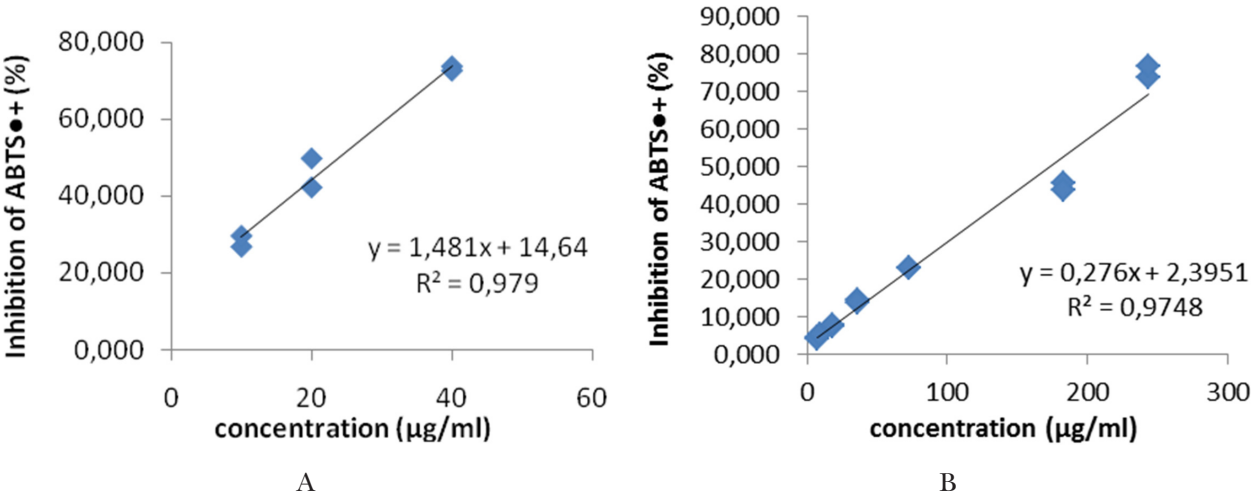
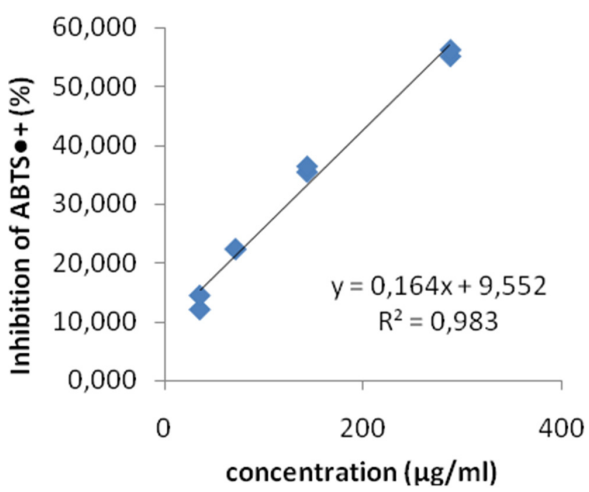


Fig. 3. The ability of A — quercetin, B — trolox scavenges ABTS radical cation. IC_{50} value of quercetin is (24 ± 1) µg/ml. IC_{50} value of trolox is (170.1 ± 0.1) µg/ml. Results are means ± SD (n = 2).

existence of hydrophilic outer surface of the cyclodextrin molecule (Yadav et al., 2009). On the other hand, according to Zaveri et al. (2011) complex of curcumin with phospholipid showed almost 60 % greater permeation of curcumin through rat skin as compared to that of plain curcumin. Phospholipid complexation of curcumin with phospholipid results in increased transdermal penetration of curcumin.

The chemical structure of curcumin plays a pivotal role in its biological activity. In addition to enhancing the amount of bioavailable curcumin researchers hope to achieve improved biological activity of curcumin by structural modifications. In the literature, numerous studies dealing with the enhancement biological activity of curcumin derivatives and analogues can be found (Anad et al., 2007). In our study (Fig. 3 and 4), there were significant differences ($P > 0.05$) in the ABTS radical cation scavenging activity among tested samples.

Their antioxidant activity decreased as follows: quercetin > trolox > curcuminoids > curcumin-cyclodextrin complex > curcumin-phospholipid complex. As can be seen, the antioxidant potential of single curcuminoids is higher than curcuminoids in complexes with cyclodextrin and phospholipid, respectively. The antioxidant mechanism of curcumin was explained by the density functional theory with five different mechanisms including single electron transfer, radical adduct formation, H atom transfer from neutral curcumin, H atom transfer from deprotonated curcumin and sequential proton loss electron transfer (Amalraj et al., 2016). In our study, we did not observed an increase in the antioxidant activity of curcumin in complexes. For



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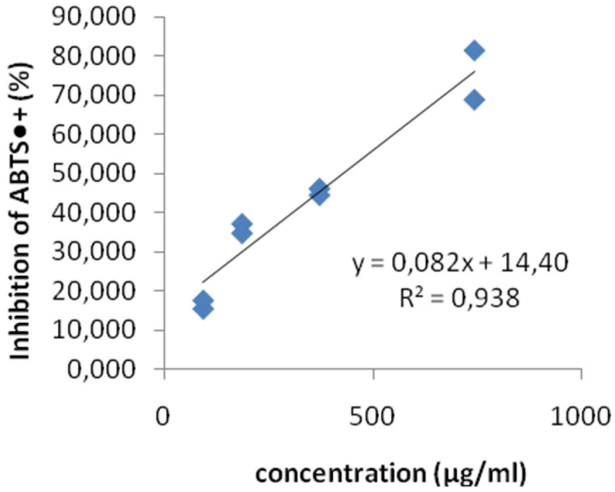

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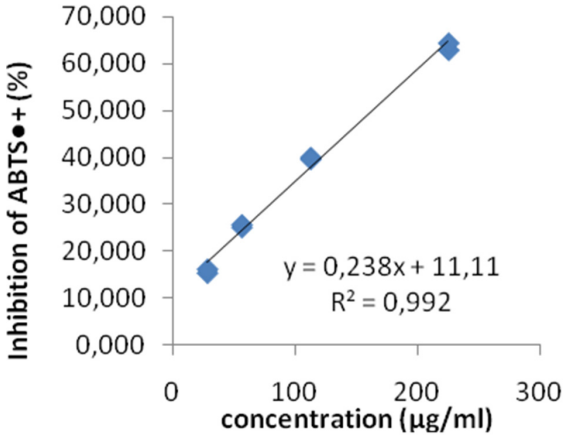

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Fig. 4. The ability of C — curcumin-cyclodextrin complex, D — curcumin-phospholipid complex, E — curcuminoids scavenges ABTS radical cation. IC_{50} value of curcumin-cyclodextrin complex is (246 ± 1) µg/ml. IC_{50} value of curcumin-phospholipid complex is (430 ± 1) µg/ml. IC_{50} value of curcuminoids is (163.4 ± 0.6) µg/ml. Results are means ± SD (n = 2).

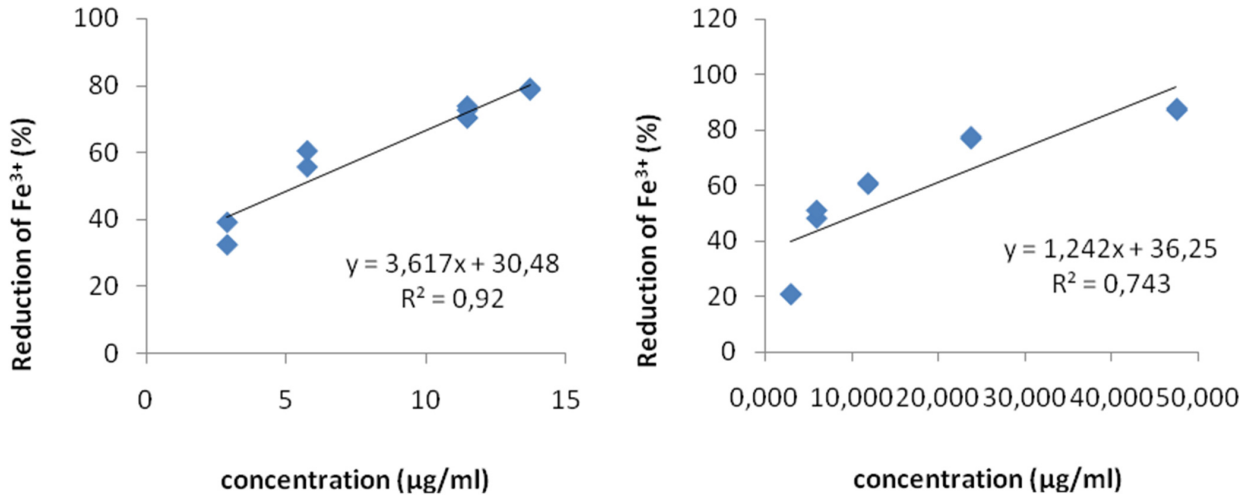

Fig. 5. The ability of A — quercetin, B — trolox to reduce Fe^{3+}. IC_{50} value of quercetin is 5.40 µg/ml ± 0.03. IC_{50} value of trolox is 11.1 µg/ml ± 0.7. Results are means ± SD (n = 2).

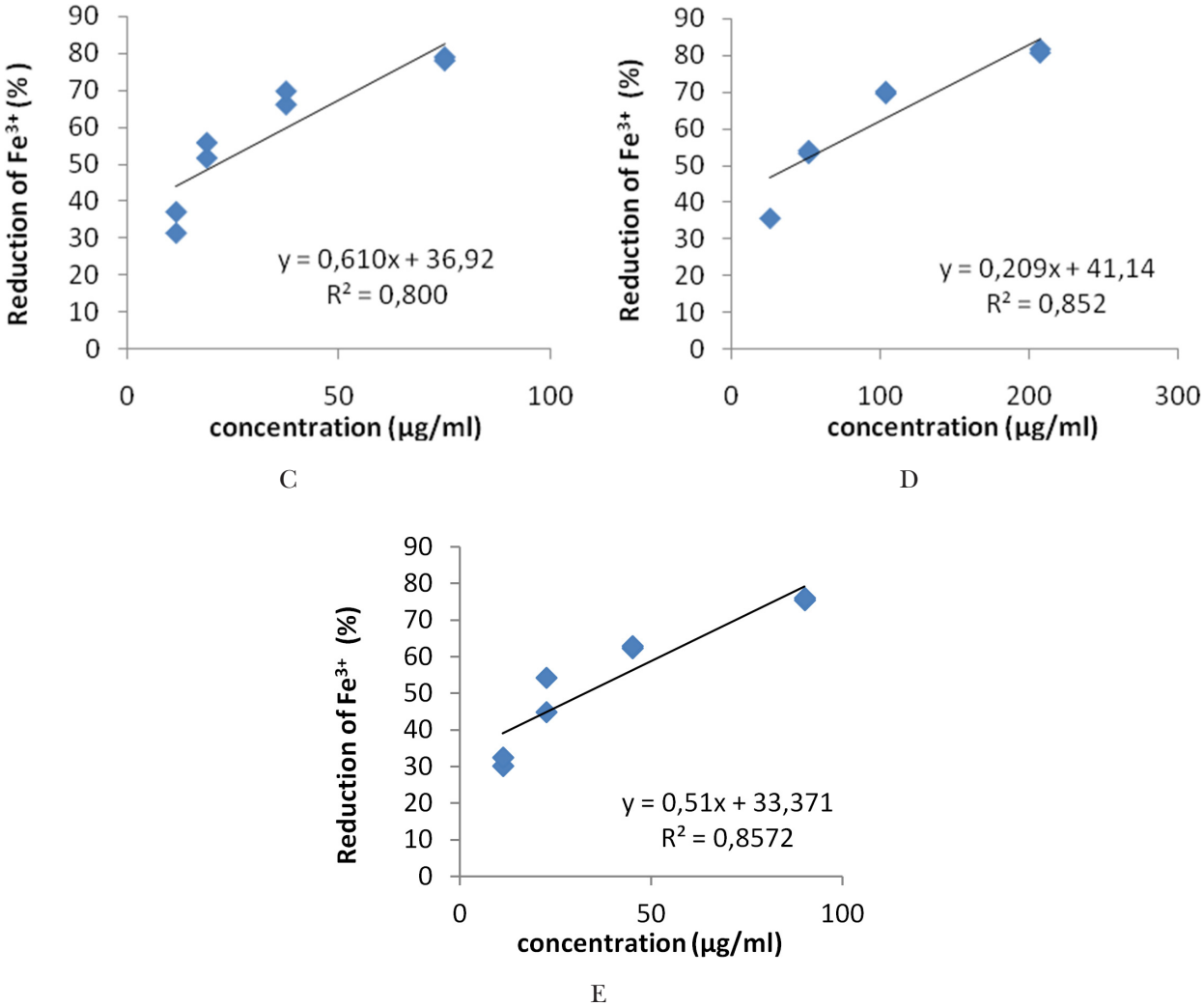

Fig. 6. The ability of C — curcumin-cyclodextrin complex, D — curcumin-phospholipid complex, E — curcuminoids to reduce Fe^{3+}. IC_{50} value of curcumin-cyclodextrin complex is 21.4 µg/ml ± 0.7. IC_{50} value of curcumin-phospholipid complex is 42.3 µg/ml ± 0.5. IC_{50} value of curcuminoids is 32.6 µg/ml ± 2.3. Results are means ± SD (n = 2).

comparison, there are few studies dealing with the antioxidant activity of curcumin complexes with cyclodextrin and phospholipid, respectively. The best ability to reduce ABTS radical cation showed quercetin. Its IC_{50} value (24 µg/ml ± 1) was 7 times lower than trolox.

The similar trend was also observed in FRAP test (Fig. 5 and 6). The reducing potential of tested samples in descending order was quercetin > trolox > curcumin-cyclodextrin complex > curcuminoids > curcumin-phospholipid complex. Our results showed that curcumin and its complexes have a capacity for iron binding. This assay indicated that curcumin has chelating activity and is able to absorb ferrous ion (Ak and Gülçin, 2008). The best reduction power showed quercetin (IC_{50} value is 5.40 µg/ml ± 0.03) and trolox (IC_{50} value is 11.1 µg/ml ± 0.7). Quercetin reached 4 times more reducing potential than curcumin-cyclodextrin complex and 6 times more than curcumin. The reducing ability of trolox was 3 times higher than that of curcumin. These results confirmed results of Ak and Gülçin (2008) who measured among other compounds the reducing power of curcumin. In their study, curcumin showed a lower ability to reduce Fe^{3+} ion than trolox or tocopherol.

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