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Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (*Curcuma longa* Linn.) ☆

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A B S T R A C T

The phytoconstituents of essential oil and ethanol oleoresin of fresh and dry rhizomes of turmeric (*Curcuma longa* Linn.) were analyzed by GC–MS. The major constituents were aromatic-turmerone (24.4%), alpha-turmerone (20.5%) and beta-turmerone (11.1%) in fresh rhizome and aromatic-turmerone (21.4%), alpha-santalene (7.2%) and aromatic-curcumene (6.6%) in dry rhizome oil. Whereas, in oleoresins, the major components were alpha-turmerone (53.4%), beta-turmerone (18.1%) and aromatic-turmerone (6.2%) in fresh and aromatic-turmerone (9.6%), alpha-santalene (7.8%) and alpha-turmerone (6.5%) in dry rhizome. Results showed that alpha-turmerone, a major component in fresh rhizomes is only minor one in dry rhizomes. Also, the content of beta-turmerone in dry rhizomes is less than a half amount found in fresh rhizomes. The antioxidant properties have been assessed by various lipid peroxidation assays as well as DPPH radical scavenging and metal chelating methods. The essential oil and ethanol oleoresin of fresh rhizomes have higher antioxidant properties as compared dry ones.

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1. Introduction

Lipids are rich source of energy and constitute an important part of our balanced diet. However, oxidation with atmospheric oxygen and lipolysis are responsible for the rancidity of lipids and lipid containing food products (Allen and Hamilton, 1983). Lipid peroxidation causes a decrease in nutritional value, safety and appearance of foods. It also initiates other undesirable changes in food, affecting its nutritional quality, color, flavor and texture. Auto-oxidation of polyunsaturated lipids involves a free radical chain reaction, generally initiated by exposure to light, heat, metal ions, etc. Therefore, the inhibition of free radical oxidation by incorporating antioxidants is of great practical importance in preserving lipids from deterioration.

Antioxidants have been widely used as additive to provide protection against oxidative degradation of foods (Gulcin et al., 2004). Although many synthetic chemicals, such as phenolic compounds are found to be strong radical scavengers, they usually have serious side effects (Imaida et al., 1983). In view of this, antioxidant substances obtained from natural sources will be of great interest. There are many herbs and spices which have been used for long ago in folk medicines. *Curcuma longa* Linn. (Family: Zingiberaceae), commonly known as turmeric, is one such perennial herb. Its rhizomes and oils have great importance. It is extensively used as spice in domestic cooking. In combination with other natural dyes, it is also used as a coloring agent for textiles, pharmaceuticals, confectionary and cosmetics (Singh et al., 2003). In Indian system of medicine, turmeric rhizomes are used in stomachache, as a blood purifier, carminative, appetizer and tonic. Turmeric is also used in drugs against cancer, dermatitis, AIDS and high cholesterol level (Kuttan et al., 1985; Azuine and Bhide, 1992; Ammon and Wahl, 1991). The essential oil extracted from turmeric also possesses anti-inflammatory, antifungal, antihepatotoxic and antiarthritic activities (Arora et al., 1971; Behura et al., 2000; Kiso et al., 1983; Palasa et al., 1992).

The objective of our work was to assess and compare the chemical components and antioxidant properties of essential oils and oleoresins of fresh and dry rhizomes of *C. longa*. In the present work we have made an attempt to assess the dietary benefits of these both rhizomes.

2. Experimental

2.1. Chemicals and reagents

All the chemicals and solvents used are of analytical grade. Butylated hydroxyanisole (BHA), butylated hydroxytoluene

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(BHT), propyl gallate (PG), glacial acetic acid, potassium ferricyanide and absolute alcohol of s.d. fine-chemicals Ltd., Mumbai, India; thiobarbituric acid (TBA), diphenyl picrylhydrazyl (DPPH) radical, linoleic acid of Fluka chemicals and NaOH, chloroform, ethyl acetate, methanol, ethanol and potassium iodide of Merk, Mumbai, India, were used as received.

2.2. Extraction of essential oils and oleoresins

The completely mature fresh rhizomes of *C. longa* were collected from local farmers during April and the same rhizomes after drying were taken as dry spice. Voucher specimens were deposited at the Herbarium of the Science Faculty of DDU Gorakhpur University, Gorakhpur, India. The fresh rhizomes were washed, air dried and thinly grated while the dry rhizomes were washed, sun dried and pulverized into a fine powder. 100 g of both types of rhizome preparations were subjected to hydrodistillation, separately, in a Clevenger's type apparatus for 5 h according to European Pharmacopoeian (1983) procedure. The light yellow colored oil obtained (yield 1.4% for fresh and 2.9% for dry rhizomes) was dried over minimum amount of anhydrous sodium sulfate and stored at 4 ± 1 °C.

Oleoresins were obtained by extraction of prepared rhizomes with ethanol. For this, 30 g of spice was loaded on the Soxhlet's apparatus and extracted with the solvent for 5–6 h. After complete extraction, the solvents were distilled off to obtain viscous oleoresins, which were stored at 4 ± 1 °C.

2.3. Chemical investigations

Chemical composition of essential oil and oleoresins of *C. longa* were analyzed by GC–MS technique using a Hewlett–Packard gas chromatograph (Model 6890) coupled with a quadruple mass spectrometer (Model HP 5973) and a Perkin Elmer Elite-5MS capillary column (5% phenylmethylsiloxane; length 30 m × inner diameter 0.25 mm × film thickness 0.25 μm). The injector, interphase, ion source and selective mass detector temperatures were maintained at 280 °C, 280 °C, 230 °C and 150 °C, respectively. Helium (He) was used as a carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed as follows:

For essential oil: at 60 °C for 1 min; then increased from 60 to 185 °C at the rate of 1.5 °C/min and held at 185 °C for 1 min; then again increased from 185 to 275 °C at the rate of 9 °C/min and held at 275 °C for 2 min.

For oleoresin of dry rhizome: at 60 °C for 1 min; then increased from 60 to 185 °C at the rate of 1.5 °C/min and held at 185 °C for 1 min; then again increased from 185 to 275 °C at the rate of 9 °C/min and held at 275 °C for 2 min.

For oleoresin of fresh rhizome: at 80 °C for 0 min; then increased from 80 to 280 °C at the rate of 10 °C /min and held at 280 °C for 40 min.

2.4. Identification of components

The components were identified on the basis of comparison of their retention indices and mass spectra with the published data (Qin et al., 2007) and computer matching was done with the Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC–MS systems. The retention indices were calculated using a homologous series of *n*-alkanes C_8–C_{18}.

2.5. Antioxidant properties

2.5.1. Lipid peroxidation assays

2.5.1.1. Sample preparation. The essential oils and oleoresins extracted from fresh and dry rhizomes of *C. longa* were added individually to unrefined crude mustard oil samples (30 g each) at the concentration of 200 ppm (w/v). Synthetic antioxidants such as BHA and BHT were also added to mustard oil at the same concentration. Mustard oil without any additive was taken as control sample. All the samples were exposed to accelerated oxidation by incubating at 70 °C in darkness. The extents of oxidation of various samples were assessed periodically by various lipid peroxidation assays.

2.5.1.2. Peroxide value. This parameter measures the total peroxide and hydroperoxide oxygen content of the mustard oil samples. The peroxide value was measured at regular intervals of 7 days during the incubation periods of 28 days, according to the procedure prescribed by Hortwitz (2002). A 5 g of mustard oil sample was dissolved in 30 mL of glacial acetic acid–chloroform (3:2) solution and mixed with 0.5 mL of saturated KI solution. After 1 min, 30 mL of distilled water was added and the mixture was titrated with 0.01 N $Na_2S_2O_3$ using starch indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. The peroxide value (Meq of peroxide/kg of oil) was calculated as:

$$\text{Meq of peroxide/kg of oil} = \frac{S \times N \times 1000}{\text{Wt of sample (g)}}$$

where S is mL of $Na_2S_2O_3$ consumed, and N is the normality of $Na_2S_2O_3$.

2.5.1.3. TBA value. The test was performed at regular intervals during the incubation periods of 28 days according to the method previously reported (Pokorny and Dieffenbacher, 1998; Marcuse and Johansson, 1973). About 100 mg of oil sample was dissolved in 25 mL of 1-butanol, mixed thoroughly with 5.0 mL of TBA reagent (200 mg TBA in 100 mL 1-BuOH) and incubated at 95 °C for 2 h. After that, the reaction mixture was cooled up to room temperature under running water and absorbance was measured at 530 nm. At the same time, a reagent blank test (without TBA reagent) was also parallely done. The TBA value (Meq of malondialdehyde/g) was calculated as:

$$\text{TBA value} = \frac{50 \times (A - B)}{M}$$

where A is the absorbance of test sample, B is the absorbance of reagent blank and M is the mass of the sample (mg).

2.5.2. Complementary antioxidant assays

2.5.2.1. DPPH free radical scavenging activity. The radical scavenging capacity of essential oils and oleoresins was monitored by measuring their ability to scavenge the DPPH radical by the method reported earlier (Cuendet et al., 1997). For this, 1 mL of freshly prepared DPPH radical solution (0.1 mM in methanol) was mixed thoroughly with 3 mL of methanolic solution of essential oil, oleoresins and synthetic antioxidants (5–20 μg/mL). The reaction mixture was left for 30 min in dark at room temperature after which the resultant absorbance was recorded at 517 nm. Control (without any additive) and standards (containing BHA, BHT and PG; in place of oil and oleoresins) were also tested. The capability to scavenge the DPPH radical (% inhibition) was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \{1 - (A_t - A_b/A_c)\} \times 100$$

where A_t is the absorbance of test sample, A_b is the absorbance of blank and A_c is the absorbance of control sample.

2.5.2.2. Ferrous ion chelating activity. The method reported by Senevirathne et al. (2006) was used to determine the ferrous ion chelating activity of different *C. longa* essential oil and oleoresins. A

200 μL amount of each essential oil/oleoresins was mixed with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine solutions. After a 10 min equilibrium period, the absorbance at 562 nm was recorded. A complex of Fe^{2+}/ferrozine showed strong absorbance at 562 nm.

2.6. Statistical analysis

Experimental results were the means ± standard deviation of three parallel measurements (data are not shown). The results were analyzed by ANOVA by using Microsoft Excel statistical analysis programme and $p \leqslant 0.05$ was considered as significant.

3. Results and discussion

3.1. Chemical investigations

A detailed and careful interpretation of the experimental data (EM fragmentation and retention indices) obtained from GC–MS analysis resulted in the identification of a large number of components in the essential oils and oleoresins of fresh and dry rhizomes of *C. longa* (Tables 1 and 2).

Table 1 shows identification of 38 components in fresh rhizome oil representing about 73.1% of the total amount and 38 components in dry rhizome oil constituting about 79.9% of the total weight. The major components were aromatic-turmerone (24.4%), alpha-turmerone (20.5%) and beta-turmerone (11.1%) in fresh rhizome oil and aromatic-turmerone (21.4%), alpha-santalene (7.2%) and aromatic-curcumene (6.6%) in dry rhizome oil. These findings are in good agreement with previous reports in which ar-turmerone and beta-turmerone were identified as major components (Zaeoung et al., 2005; Martins et al., 2001; Gopalan et al., 2000). Many other workers also reported similar results with respect to the content of major constituents (Singh et al., 2003; Jayaprakasha et al., 2005; Riaz et al., 2000; Nigam and Ahmad, 1990; Sharma et al., 1997).

Table 2 shows the chemical composition of ethanol oleoresins from the two types of rhizomes. A total of 13 components were identified in fresh rhizome representing about 82.3% and 28 components were identified in dry rhizome representing about 69.7%

Table 1
Chemical composition of the essential oil of fresh (FC1) and dry (DC1) rhizomes from *C. longa*.

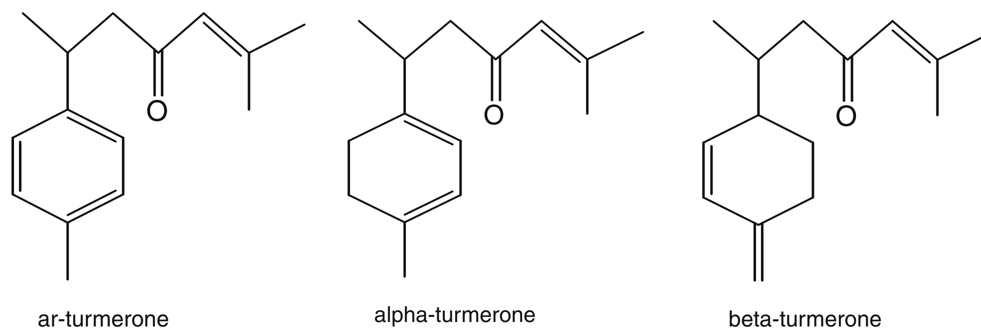
Compound	FC1 (%)	DC1 (%)	Identification[a]
alpha-Pinene	Trace	Trace	MS, RI, co-GC
Sabinene	Trace	Trace	MS, RI, co-GC
beta-Pinene	Trace	Trace	MS, RI, co-GC
Myrcene	0.1	Trace	MS, RI, co-GC
alpha-Phellandrene	0.1	–	MS, RI, co-GC
3-Carene	0.1	–	MS, RI, co-GC
alpha-Terpinene	0.1	–	MS, RI, co-GC
p-Cymene	0.3	0.1	MS, RI, co-GC
Limonene	0.1	Trace	MS, RI, co-GC
1,8-Cineole	0.4	0.1	MS, RI, co-GC
Terpinolene	2.7	Trace	MS, RI, co-GC
p-Cymen-8-ol	0.9	Trace	MS, RI, co-GC
beta-Elemene	–	0.1	MS, RI
cis-alpha-Bergamotene	0.1	0.2	MS, RI
beta-Caryophyllene	3.1	0.5	MS, RI
alpha-Santalene	0.4	7.2	MS, RI
trans-alpha-Bergamotene	0.1	1.6	MS, RI
epi-beta-Santalene	Trace	1.0	MS, RI
alpha-Humulene	0.6	–	MS, RI
trans-beta-Farnesene	0.2	0.8	MS, RI
Sesquisabinene	0.2	1.6	MS, RI
ar-Curcumene	1.6	6.6	MS, RI
alpha-Zingiberene	2.5	0.8	MS, RI
(E,E)-alpha-Farnesene	Trace	0.1	MS, RI
beta-Bisabolene	0.8	4.1	MS, RI
Sesquicineole	–	0.1	MS, RI
beta-Sesquiphellandrene	2.9	4.2	MS, RI
trans-gamma-Bisabolene	0.1	0.9	MS, RI
cis-Sesquisabinene hydrate	0.3	0.6	MS, RI
trans-Nerolidol	0.5	0.3	MS, RI
Santalenone	1.1	5.6	MS, RI
ar-Turmerol	0.4	0.9	MS, RI
cis-beta-Elemenone	–	0.1	MS, RI
Dihydro-ar-turmerone	0.4	0.9	MS, RI
ar-Turmerone	24.4	21.4	MS, RI
alpha-Turmerone	20.5	0.6	MS (Ref. 1)
beta-Bisabolol	–	3.0	MS, RI
Germacrone	1.0	2.6	MS, RI
beta-Turmerone	11.1	4.3	MS (Ref. 1)
Curcuphenol	0.2	–	MS, RI
6R, 7R-Bisabolone	1.7	0.8	MS, RI
trans-alpha-Atlantone	0.9	2.6	MS, RI
Total	79.9%	73.1%	

Trace: <0.05%; # the retention index (RI) was calculated using a homologous series of *n*-alkanes C_8–C_{18}.
Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.
[a] Co-GC: co-injection with an authentic sample.

Table 2
Chemical composition of the ethanol oleoresin of fresh (FC2) and dry (DC2) rhizomes from *C. longa*.

Compound	FC2 (%)	DC2 (%)	Identification[a]
ar-Curcumene	Trace	4.2	MS, RI
alpha-Zingiberene	Trace	6.1	MS, RI
beta-Sesquiphellandrene	Trace	6.9	MS, RI
trans-Nerolidol	Trace	–	MS, RI
Santalenone	0.2	4.7	MS, RI
ar-Turmerol	Trace	–	MS, RI
Dihydro-ar-turmerone	0.9	–	MS, RI
ar-Turmerone	6.2	9.6	MS, RI
alpha-Turmerone	53.4	6.5	MS
Germacrone	0.7	1.4	MS, RI
beta-Turmerone	18.1	4.3	MS
6R, 7R-Bisabolone	2.0	0.7	MS, RI
trans-alpha-Atlantone	0.8	2.6	MS, RI
Palmitic acid	–	–	MS
Stigmasterol	–	–	MS
Sitosterol	–	–	MS
Stigmast-4-en-3-one	–	–	MS
1,8-Cineole	–	0.2	MS, RI, co-GC
Terpinen-4-ol	–	Trace	MS, RI, co-GC
p-Cymen-8-ol	–	Trace	MS, RI, co-GC
alpha-Terpineol	–	Trace	MS, RI, co-GC
beta-Elemene	–	–	MS, RI
7-epi-Sesquithujene	–	0.3	MS, RI
cis-alpha-Bergamotene	–	0.2	MS, RI
beta-Caryophyllene	–	–	MS, RI
alpha-Santalene	–	7.8	MS, RI
trans-alpha-Bergamotene	–	1.4	MS, RI
epi-beta-Santalene	–	1.1	MS, RI
trans-beta-Farnesene	–	2.7	MS, RI
beta-Santalene	–	0.2	MS, RI
Sesquisabinene	–	–	MS, RI
gamma-Curcumene	–	0.2	MS, RI
(E,E)-alpha-Farnesene	–	0.2	MS, RI
beta-Bisabolene	–	4.6	MS, RI
beta-Curcumene	–	0.5	MS, RI
Sesquicineole	–	Trace	MS, RI
trans-gamma-Bisabolene	–	0.9	–
cis-Sesquisabinene hydrate	–	–	MS, RI
cis-beta-Elemenone	–	–	MS, RI
beta-Bisabolol	–	2.4	MS, RI
Total	82.3%	69.7%	

Trace: <0.05%; # the retention index (RI) was calculated using a homologous series of *n*-alkanes C_8–C_{18}.
Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.
[a] Co-GC: co-injection with an authentic sample.



Scheme 1. Major constituents of essential oil and oleoresin of *C. longa* rhizomes.

of the total weight. The major components were alpha-turmerone (53.4%), beta-turmerone (18.1%) and aromatic-turmerone (6.2%) in fresh rhizome and aromatic-turmerone (9.6%), alpha-santalene (7.8%) and alpha-turmerone (6.5%) in dry rhizome. Mara et al. (2003) reported ar-turmeronol and (Z)-alpha-alantone as the major components in the ethanol extract of turmeric rhizomes (Scheme 1).

It is interesting to note that alpha-turmerone, a major component in the essential oil (20.5%) and ethanol oleoresin (53.4%) from fresh rhizome is only a very minor one in the essential oil (0.6%) and oleoresin (6.5%) of the dry rhizome. Also, the content of beta-turmerone in dry rhizome (4.3% in essential oil as well as oleoresin) is less than a half of the amount found in green rhizomes (11.1% in essential oil and 18.1% in the oleoresin). In dry rhizome, comparatively less percentage of alpha- and beta-turmerone may be probably due to, absence of aromatic ring and presence of two conjugated double bonds which may undergo oxidation/polymerization very easily.

3.2. Antioxidant properties

The amount of peroxides and hydroperoxides formed during the initial stages of lipid peroxidation are determined by measuring their peroxide value (PV). Fig. 1 shows the PV for all the tested antioxidative substances. It was observed that all the tested substances showed significantly lower peroxide content than the control for which PV increased from 26.7 Meq/kg (day 1) to 302.9 Meq/kg (day 28). However, other samples showed lesser peroxide content. The lowest peroxide level was shown by the sample containing essential oil from fresh rhizomes and the second lowest PV was observed for the sample with essential oil of dry rhizome. The relative efficacies of various test substances were; essential oil of fresh rhizome > essential oil of dry rhizome > ethanol oleoresin of fresh rhizome > ethanol oleoresin of dry rhizome > BHT > BHA > control.

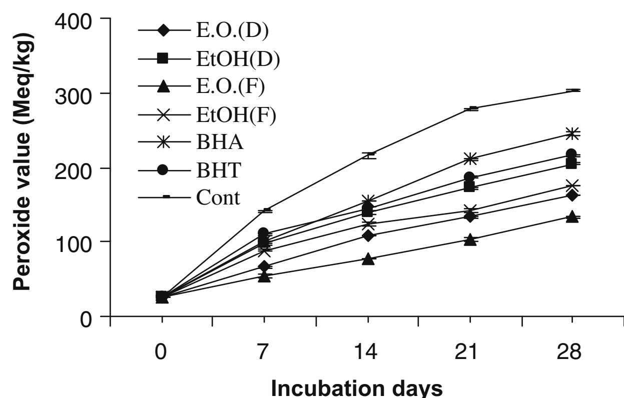


Fig. 1. Antioxidant activity of *C. longa* oil and oleoresins in terms of peroxide values (D = dry; F = fresh).

This assay measures the inhibitory effect of various antioxidative substances on the formation of malondialdehyde, a secondary oxidation product. These results (Fig. 2) clearly show that essential oils of both fresh and dry rhizomes were more effective than synthetic antioxidants while the activities of oleoresins were comparable to BHT but higher than BHA. Moreover, essential oil and

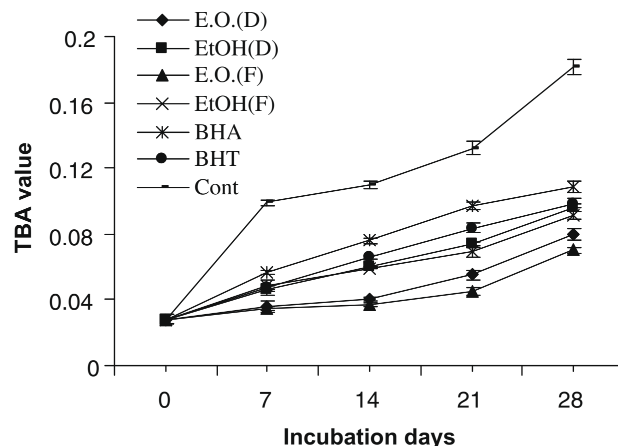


Fig. 2. Antioxidative effect of *C. longa* oil and oleoresins in terms of thiobarbituric acid values.

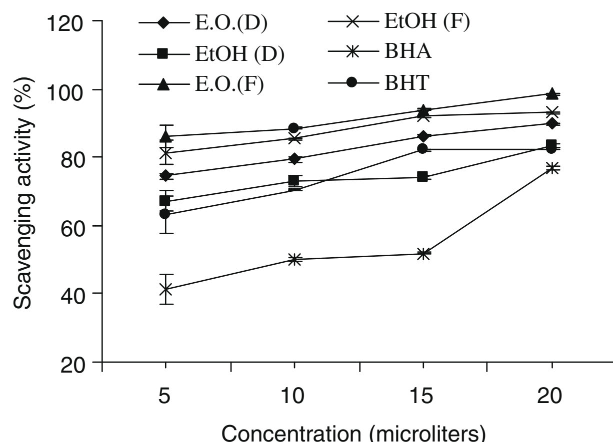


Fig. 3. Radical scavenging effect of *C. longa* essential oil and oleoresins on 2,2′-diphenyl-1-picrylhydrazyl radical.

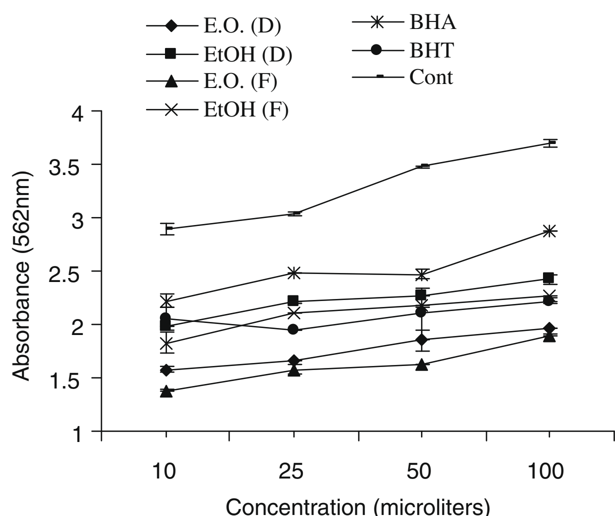


Fig. 4. Fe^{2+} chelating effect of *C. longa* essential oil and oleoresins.

oleoresin from fresh rhizomes were found to be more effective than those from dry rhizomes.

The radical scavenging property of essential oils and oleoresins were tested by measuring their ability to scavenge DPPH radicals. All the tested substances showed a dose dependent radical scavenging activity (Fig. 3). The essential oil of fresh rhizomes showed the strongest activity followed by its oleoresin and then by essential oil and oleoresin of dry rhizomes, respectively. The activity of all the four essential oils and oleoresins were significantly higher ($p < 0.05$) than the BHA while the activity of dry rhizome oleoresin is comparable to that of BHT. Zaeoung et al. (2005) also reported the strong antioxidant activity of methanol extract of *C. longa* against the DPPH radical with % inhibition in the range of 86–92%.

The ferrous ion chelating activities of the tested antioxidative substances are shown in Fig. 4. Ferrozine can quantitatively form complex with Fe^{2+} but in the presence of ion chelating substances, the complex formation is disrupted, resulting in a decrease in the red color of the complex. Among the tested substances, essential oil of fresh rhizomes showed the highest Fe^{2+} chelating ability which was significantly higher ($p < 0.05$) than BHA and BHT. Second highest activity was shown by essential oil of dry rhizomes which was also significantly higher ($p < 0.05$) than the values of commercial antioxidants. Further, oleoresin of both types of rhizomes showed chelating activity equivalent to BHA but lower than BHT. In addition, the essential oil and oleoresin of fresh rhizome were better chelators than the essential oil and oleoresin from dry rhizome.

The active principle in turmeric is a group of phenolic compounds including curcumin which is very well known for its antioxidant activity (Miquel et al., 2002). Zaeoung et al. (2005) and Masuda et al. (2001) have reported the strong antioxidant activity of curcuminoids from turmeric. In the present investigation we reported that turmeric oil and oleoresin are also effective antioxidants. ar-Turmerone and alpha-turmerone are the major constituents in essential oil and oleoresin. Probably, these compounds exert either the synergistic or additive actions towards the total antioxidant activity. It may also be possible that these compounds alone or in synergy with other compounds (viz., curcumene, santalene, sitosterol, etc.) present in the essential oil and oleoresin are responsible for the observed antioxidant activity of rhizomes of *C. longa*.

Results showed that essential oil and oleoresin of fresh rhizomes of *C. longa* showed higher antioxidant properties as compared to dry ones. A comparison of the same species in its two different physical states of rhizomes-fresh and dry, showed considerable loss in antioxidant properties in dry state.

From GC–MS data, it is clear that in dry rhizomes' essential oil and oleoresin, the amount of alpha- and beta-turmerone is considerably less than that of the fresh rhizomes in which these are the major constituents. The higher activity of fresh rhizomes might be due to the presence of these compounds in major quantity. Loss of antioxidant properties during the drying thus signifies that its beneficial pharmacological activities were definitely reduced. Thus, fresh turmeric rhizomes should be preferred for domestic, culinary and other purposes.

Conflict of interest

The authors declare that there are no conflicts of interest.

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