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# Comparison of chemical composition and antioxidant potential of volatile oil from fresh, dried and cured turmeric (*Curcuma longa*) rhizomes

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ABSTRACT

The present work was conducted to assess and compare the chemical composition of volatile oils from fresh, dried and cured turmeric (*Curcuma longa*) rhizomes from a selected single source. In addition, their antioxidant and radical scavenging potentials were correlated with chemical composition. Major components were *ar*-turmerone (21.0–30.3%), $\alpha$-turmerone (26.5–33.5%) and $\beta$-turmerone (18.9–21.1%). Trolox equivalent antioxidant capacity (TEAC) values were 38.9, 68.0 and 66.9 μM at 1 mg of oil/ml for fresh, dried and cured rhizome respectively in ABTS assay. $IC_{50}$ values for fresh, dried and cured rhizome oil to quench DPPH radicals were 4.4, 3.5 and 3.9 mg of oil/ml respectively. Fresh, dried and cured rhizome oils showed antioxidant capacity of 358, 686 and 638 mM of ascorbic acid equivalents per 1 mg of oil respectively. The rhizome oil shows good reducing potential and was concentration dependent. It is inferred that the cured rhizomes provided high yield of volatile oil with appreciably high antioxidant potential.



## 1. Introduction

Turmeric is the rhizome of the plant *Curcuma longa* Linn., a tropical herb of the zingiberaceae family and native to southern Asia. It is primarily consumed in the form of powdered rhizomes mainly for coloring because of its yellow color and its associated medicinal properties. It imparts characteristic flavor and preserves the freshness of the product prepared (Govindarajan and Stahl, 1980). The curcuminoid pigments and volatile oils, which are the major secondary metabolites of turmeric, have been shown to be largely responsible for the pharmacological activities of turmeric powder, extracts and oleoresins. Turmeric oleoresins commonly contain curcuminoid pigments (diarylheptanoids; 30–45%) and volatile oil (15–20%); the volatile oil contains turmerone (60%), $\alpha$-zingiberene (25%) and small quantities of $\alpha$-phellandrene, sabinene, eucalyptol, borneol and sesquiterpenes (Sasikumar, 2001). Turmeric owes its aromatic taste and smell due to the volatile oil present in the rhizome. The volatile oil obtained by hydrodistillation of turmeric rhizomes grown in the North Indian Plains contains eucalyptol (11.2%), $\alpha$-turmerone (11.1%), $\beta$-caryophyllene (9.8%), *ar*-turmerone (7.3%) and $\beta$-sesquiphellandrene (7.1%) as major constituents (Raina et al., 2002).

The volatile oil was isolated from turmeric rhizome and was analyzed by GC–MS and reported to contain 16 constituents of which, 6 compounds contributing 70.0% of the total oil constituents (Naz et al., 2010). The major components present were *ar*-turmerone (25.3%), $\alpha$-turmerone (18.3%), $\beta$-turmerone (12.5%), $\beta$-caryophyllene (2.26%), eucalyptol (1.60%) and $\alpha$-phellandrene (0.42%). The turmeric oil obtained by supercritical fluid extraction under optimized conditions has been reported to yield a high amount of *ar*-turmerone, $\alpha$-turmerone and $\beta$-turmerone (Manzan et al., 2003). The essential oil from the rhizome of turmeric grown in Pakistan was isolated by the hydro-distillation in which 17 components were identified and reported (Naz et al., 2011). The main components of the essential oil were *ar*-turmerone (38.59%), $\alpha$-turmerone (8.88%) and $\beta$-turmerone (12.9%). The rhizome oils of *Curcuma angustifolia* from central and southern India were isolated and subjected to GC/MS analysis (Srivastava et al., 2006). It was reported that xanthorrhizol isomer (12.7%), methyleugenol (10.5%), palmitic acid (5.2%) and camphor (4.2%) were the major constituents of the rhizome oil from the central India, while the rhizome oil from southern India contained germacrone (12.8%), camphor (12.3%), isoborneol (8.7%), curdione (8.4%) and eucalyptol (4.8%) as major constituents.

In recent years, the evaluation of antioxidant potential of foods has received much attention. Much work has been carried out on the antioxidant and related anticancer activities of compounds (especially curcuminoids) derived from turmeric rhizomes. In ayurvedic medicinal system, turmeric has been extensively used in both fresh and dried forms internally as a stomachic, tonic and blood purifier and externally in the prevention and treatment of skin diseases (The Wealth of India, 2001). The main activities of

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curcuma rhizome have been found to be anti-inflammatory, hepatoprotective, anti-microbial, antifungal, antiviral, wound healing, anticancer, antitumor, anti-inflammatory and antivenom agents (Jayaprakasha, 2005; Negi et al., 1999; Raina et al., 2002). Essential oils of different biological origin were extracted by steam distillation and its chemical composition and antioxidant properties were evaluated and compared (Sacchetti et al., 2005). They reported that the essential oil of turmeric rhizome showed major radical scavenging activity against DPPH free radical. Singh et al. (2010) studied the antioxidant activities of essential oil and oleoresin extracted from fresh and dried rhizomes of *C. longa* and reported that the essential oil and ethanol oleoresin from fresh rhizomes showed high antioxidant activity than the dried ones.

The turmeric rhizome is commercially available as a whole cured rhizome (fresh rhizomes are cooked in water, dried in shade and polished) and its powdered form for culinary purpose. In ayurveda, fresh turmeric rhizomes as well as turmeric in powdered form are used for the preparation of medicinal formulations. The comparative data of the chemical composition of volatile oil obtained from fresh, dried and cured rhizomes and their biological activities from a single source, single geographical location and same season in a year are scarce. Hence, scope exists to extract volatile oil from fresh, dried and cured rhizomes and to analyze their chemical composition and antioxidant potential. Correlation of antioxidant potential of the volatile oil with its chemical composition will give an insight into its potential use.

The objective of the present study is to extract volatile oil from fresh, dried and cured rhizomes of turmeric from a single source, single geographical location and same season in a year and to compare their chemical composition. In addition, the radical scavenging and antioxidant potential of the volatile oils were evaluated by standard assays and correlated with their chemical composition.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), L-ascorbic acid, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), homologous series of $C_8$–$C_{26}$ *n*-alkane standards and reference standards ($\alpha$-pinene, myrcene, *p*-cymene, 1,8-cineole, limonene, $\alpha$-terpineol and thymol) were procured from Sigma (St. Louis, MO, USA). Sodium phosphate (monobasic and dibasic), anhydrous sodium sulfate, ammonium molybdate, ferric chloride anhydrous, potassium ferric-cyanide, anhydrous sodium acetate, tri-chloroacetic acid (TCA) and potassium persulfate were procured from SRL Chemicals (Mumbai, India). Acetic acid, hydrochloric acid and sulfuric acid were procured from Merck (Mumbai, India). All other chemicals, solvents and reagents used were of analytical grade.

### 2.2. Plant material

Fresh turmeric (*C. longa*) rhizomes were procured from a local cultivator near Mysore, Karnataka, India. The matured rhizomes were harvested when the plants were 10 months old. The rhizomes were manually cleaned with water to remove the adhering soil and extraneous matter, if any. Surface water was removed with a dry cloth. These cleaned fresh rhizomes were grated and subjected to blend in a laboratory blender to obtain a fine paste. This sample is referred as 'fresh' rhizome sample. The fresh rhizomes were grated and dried at 40 °C in a tray dryer for 8 h followed by powdering in a laboratory model grinder (comminuting mill, Cadmach Machinery Co. Pvt. Ltd., Ahmedabad, India) to obtain fine powder having a moisture content of 8.3 ± 0.1%; this sample has been referred as 'dried' rhizome sample. The fresh rhizomes were cured by employing the conventional method by cooking in excess of boiling water for about an hour; latter, excess water was discarded and the rhizomes were dried in shade for a week. These dried rhizomes were powdered to obtain fine powder having a moisture content of 10.7 ± 0.2%; this sample is referred as 'cured' rhizome sample.

### 2.3. Isolation of volatile oil from turmeric rhizome

The fresh rhizome paste, dried and cured rhizome powders were subjected to hydro distillation in Clevenger distillation until all the oil is collected (8 h). The pale yellow colored oil obtained was dried over anhydrous sodium sulfate and stored at 5 °C for further analysis. Each of the samples was subjected to distillation thrice and the results were expressed as mean ± standard deviation.

### 2.4. Chemical characterization and quantification of volatile oil

#### 2.4.1. Chemical characterization of volatile oil

The chemical composition of volatile oil obtained was analyzed using Perkin Elmer gas chromatograph equipped with quadrupole mass spectrometer (Model: Turbomass Gold) fitted with an Elite-1 fused silica column (30 m × 0.25 mm × 0.25 μm film thickness) coated with polydimethyl siloxane. Helium gas was used as carrier gas at a flow rate of 1 ml/min; injector port and detector temperatures were maintained at 250 °C; oven temperature was maintained at 50 °C for 3 min initially, and then was increased to 250 °C at the rate of 2 °C/min and was maintained at 250 °C for 3 min; ionization voltage was 70 eV. The volatile oil was diluted to 10 times with hexane and 1 μl of the resulting solution was injected to GC–MS system. Retention indices (RIs) were calculated for each compound against *n*-alkane standards ($C_8$–$C_{26}$) according to the Kovats method (Jennings and Shibamoto, 1980). The volatile oil samples were injected thrice to check the reproducibility of the data, and the results were expressed as mean ± standard deviation. The constituents of the volatile oil were identified by comparing their GC retention indices with that of the published values in literature (NIST Chemistry web book), and by comparing the mass spectral fragmentation pattern with inbuilt library provided along with instrument and also with the MS fragmentation pattern of compounds published (Adams, 2001).

#### 2.4.2. Quantification of volatile oil

The volatile oil was quantified using gas chromatograph equipped with flame ionization detector (Model # Fisons GC 8000 series) fitted with VF-1 fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) coated with polydimethyl siloxane. Oven temperature was maintained at 50 °C for 3 min initially, and then was increased to 250 °C at the rate of 4 °C/min and was maintained at 250 °C for 3 min. Injector port and detector temperatures were maintained at 250 °C. The volatile oil was diluted to 10 times with hexane and 1 μl of the resulting solution was injected. The sample was injected in triplicate under same experimental conditions. The absolute quantities of individual components were quantified by internal normalization method and expressed as mg/100 g of rhizome.

### 2.5. Antioxidant potential

In light of the differences among the wide range of test systems available, the results of a single antioxidant assay can give only a reductive suggestion of the antioxidant potential of volatile oils toward food matrices. Moreover, the chemical complexity of volatile oils with a mixture of compounds with different functional

groups, polarity and chemical behavior, could lead to a scattered result (Sacchetti et al., 2005). Therefore, an approach with multiple assays in screening work is highly desirable. Among the wide array of methods available for the evaluation of the antioxidant activity, very few of them are useful for determining the activity of both hydrophilic and lipophilic species (Frankel et al., 1994). Thus to ensure a better comparison of the results and covering a wider range of possible applications, in vitro antioxidant activity of the turmeric oil was assessed by five standard methods like free radical scavenging activity using ABTS and DPPH radical, ferric reducing antioxidant potential (FRAP) assay, total antioxidant potential using phosphomolybdenum method and reducing potential assay. The volatile oil was analyzed at different concentration levels in triplicates by each assay method and the results were expressed as mean ± standard deviation (SD).

#### 2.5.1. Radical scavenging potential of turmeric oil by ABTS radical cation assay

The free radical scavenging activity of volatile oil samples was determined using ABTS radical cation decolorization assay (Re et al., 1999). In this assay, the blue-green ABTS radical cation chromophore was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12–16 h in dark at 27 °C. The ABTS radical cation solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The resulting solution was equilibrated at 30 °C. An aliquot of 100 μl of methanolic solution of volatile oil samples (20–100 mg/ml) and standard trolox solution (10–100 μM) was taken in different test tubes. To this 1.9 ml of ABTS radical cation solution was added to make reaction mixture with the following concentrations (1–5 mg/ml) of volatile oil. The mixture was vigorously mixed in a vortex mixer for a minute, and allowed to stand at 27 °C in dark for 10 min. Reagent blank was prepared as above without addition of sample or standard. Absorbance of the mixtures was measured at 734 nm using UV–visible spectrophotometer (Model: UV-160A, Shimadzu, Japan). Results were also expressed as trolox equivalent antioxidant capacity (TEAC) and expressed as μM/mg of volatile oil.

#### 2.5.2. DPPH radical scavenging activity of turmeric oil

The scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of the volatile oil samples was determined according to the method of Blois (1958) with marginal modifications. One milliliter of methanolic solution of volatile oil (2–10 mg/ml) and standard trolox solutions (2–20 μM) was taken in different test tubes. To this solution 1 ml of 0.2 mM methanolic solution of DPPH was added to make reaction mixture with the following concentrations (1–5 mg/ml) of volatile oil. The mixture was vigorously mixed in a vortex mixer for a minute and allowed to stand at 27 °C in dark for 30 min. Reagent blank was prepared as above without addition of sample or standard. Absorbance of the mixtures was measured at 517 nm. The radical scavenging activity was calculated using Eq. (1) and expressed as percent inhibition.

$$\text{Radical scavenging activity}\,(\%) = \left[1 - \frac{A_{\text{sample}}}{S_{\text{Blank}}}\right] \times 100 \qquad (1)$$

where $A_{\text{sample}}$ and $A_{\text{blank}}$ are absorbance of sample and blank at 517 nm, respectively.

The $IC_{50}$ value (concentration of sample required to cause 50% inhibition of the free radical) was evaluated. TEAC was also calculated and expressed as trolox equivalent (μM/mg of sample).

#### 2.5.3. Ferric reducing antioxidant potential (FRAP) of turmeric oil

The total antioxidant potential of volatile oil samples was assessed using the ferric reducing ability of plasma (FRAP) assay (Benzie and Strain, 1996). The assay was based on the reducing power of a compound (antioxidant). The working FRAP reagent was prepared freshly by combining 25 ml of acetate buffer (300 mM, pH 3.6) with 2.5 ml of TPTZ solution (10 mM) in hydrochloric acid (40 mM) and 2.5 ml of ferric chloride hexahydrate solution (20 mM) in distilled water. The reagent was equilibrated at 37 °C in water bath for 5 min. An aliquot of 100 μl of methanolic solution of volatile oil samples (20–60 mg/ml) was mixed with 1.9 ml of FRAP reagent to make reaction mixture with the following concentrations (1–3 mg/ml) of volatile oil and incubated at 37 °C in water bath for 10 min. A typical reagent blank was prepared by combining 1.9 ml of FRAP reagent with appropriate volume of same solvent used for the sample. Absorbance of the resulting solution was measured at 593 nm against reagent blank. The standard calibration curve was plotted using different concentrations of standard trolox solution (50–500 μM). Results were expressed as μM trolox equivalents/mg of volatile oil.

#### 2.5.4. Total antioxidant potential by phosphomolybdenum assay

The total antioxidant capacity of volatile oil samples was evaluated by the method of Prieto et al. (1999). One milliliter of sample (50–200 μg/ml) was combined with 3 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were capped and incubated at 95 °C in water bath for 90 min. The mixtures were cooled to room temperature and the absorbance of each solution was measured at 695 nm against a typical reagent blank. The standard calibration curve was plotted using different concentrations of ascorbic acid solution (10–100 mM). The increase in absorbance of the reaction mixture shows higher antioxidant potential. The results were expressed as ascorbic acid equivalent (mM/μg of sample).

#### 2.5.5. Reducing potential

The reducing power of the volatile oil samples was determined by the method of Oyaizu (1986). Accordingly, 1 ml of volatile oil sample (2–20 mg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide solution (1%). The resulting mixture was incubated at 50 °C for 20 min and then the mixture was cooled rapidly. To this mixture 2.5 ml of trichloroacetic acid solution (10%) was added and mixed well, and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was diluted with distilled water (2.5 ml). To this solution 0.5 ml of ferric chloride solution (0.1%) was added and mixed. The absorbance of the mixture was measured at 700 nm. Increase in absorbance of the reaction mixture indicates an increase in the reduction capability of the sample.

### 2.6. Statistics and interrelation

All experiments were carried out in triplicate and expressed as mean ± standard deviation (SD). Duncan's Multiple Range Test (DMRT) was applied to determine the existence of significant difference at $p \leq 0.05$ for absolute quantities of each chemical component in the volatile oil and the antioxidant potential of turmeric oil at different concentration levels (Little and Hills, 1978).

## 3. Results and discussion

### 3.1. Volatile oils from fresh, dried and cured turmeric rhizomes

The turmeric volatile oils were isolated from fresh, dried and cured rhizomes of *C. longa* by conventional Clevenger's hydro distillation. Yield of volatile oil obtained for fresh, dried and cured rhizomes is 3.52 ± 0.23, 3.05 ± 0.15 and 4.45 ± 0.37%, respectively on a dry weight basis. The method of drying usually has a significant effect on the quality and quantity of the volatile oils (Asekun et al.,

2007). However, the yield of volatile oil obtained from cured rhizome was higher than that of fresh (26%) and dried (46%) samples. The volatile oil is present in the oil cells and ducts present in the meristematic region of the rhizome (Ravindra et al., 2007). These oil cells present are damaged during the grating of the rhizome resulting in the exposure of the oil present in the cells to atmosphere, which is lost during the drying process (Diaz-Maroto et al., 2002). The dried sample appears to be more fibrous and harder and thus it is more difficult to break and grind. Depending on the type of mill and the speed of crushing involved in the process of grinding the dried spices, the spices gets heat up and results in the loss of volatile oils (Balakrishnan, 2007). This may be a possible reason for low yield of volatile oil in dried rhizome samples. But in the case of cured sample, the rhizomes are cooked as such in boiling water and then dried in shade. In this case the oil cells are not damaged and avoid the loss during drying process. In the process of curing turmeric rhizomes, the starch granules get gelatinized due to thermal processing and this facilitates uniform drying and increases the dehydration rate (Govindarajan and Stahl, 1980). As the rhizomes show higher volumes due to cooking or gelatinization of starch present in them, the volume of cooked rhizome is more than that of uncooked sample. It must have helped in the release of total volatiles. The cured sample is easy to grind and the oil extractability is more, when compared to the uncured sample.

### 3.2. Chemical characterization and quantification of volatile oil

The chemical components present in the volatile oil were identified and presented in Table 1, according to their order of elution on Elite-1 column. The absolute quantities of the chemical components present were quantified based on GC data by internal normalization method and presented in Table 2. Totally, 28 compounds were identified in the volatile oil of fresh rhizome; most of the compounds are derived from mevalonic acid pathway biogenetically (i.e., mono and sesquiterpenoids). The major components were sesquiterpenoid i.e., *ar*-turmerone, $\alpha$-turmerone and $\beta$-turmerone whereas $\alpha$-phellandrene, $\alpha$-zingiberene, $\beta$-sesquiphellandrene, *ar*-curcumene and eucalyptol were the minor constituents. Fourteen components were identified in dried rhizome oil; the major compounds were *ar*-turmerone, $\alpha$-turmerone and $\beta$-turmerone and the minor components were $\alpha$-zingiberene, $\beta$-sesquiphellandrene and *ar*-curcumene. Most of the monoterpene hydrocarbons present in the fresh rhizome oil were not present in dried rhizome oil. The low boiling point as well as high volatility of these monoterpenes resulted in their loss during processing steps like grating, drying and grinding of the rhizome (Diaz-Maroto et al., 2002). The concentration of $\alpha$-turmerone is more in the fresh rhizome oil, when compared to that of dried rhizome oil; however, the quantity of *ar*-turmerone increased by 25% in dried rhizome oil (Table 2). This may be due to rearrangement and oxidation of less stable $\alpha$-turmerone to the most stable *ar*-turmerone (Singh et al., 2010; Su et al., 1982). In cured rhizome oil only 18 compounds were identified. However, most of the major compounds present in the fresh rhizome oil were retained whereas the minor compounds present were lost. The concentration of $\alpha$-turmerone is marginally more in the fresh rhizome oil, when compared to that of cured rhizome oils; however, the quantities of *ar*-turmerone and $\beta$-turmerone are increased by 70% and 41% respectively (Table 2). This may be due to complete release of these compounds during curing and further processing. Another reason may be due to oxidation or rearrangement of less stable $\alpha$-turmerone to the more stable *ar*-turmerone and $\beta$-turmerone (Su et al., 1982).

Chemical composition of volatiles was found to be different from other geographical locations. Raina et al. (2002) reported the presence of 84 compounds in the oil of rhizome from North Indian Plains, of which the major compounds were eucalyptol (11.2%), $\alpha$-turmerone (11.1%), $\beta$-caryophyllene (9.8%), *ar*-turmerone (7.3%) and $\beta$-sesquiphellandrene (7.1%). Jayaprakasha (2005) have reported *ar*-turmerone, zingiberene, $\alpha$-turmerone and $\beta$-turmerone as major constituents in volatile oil from spent turmeric oleoresin from South India, which compliments our findings. Volatile oil from the rhizomes of *Curcuma haritha* Mangaly and Sabu from the Western Ghats was isolated by hydro distillation and was characterized (Raj et al., 2008). Fifty compounds were identified, of which camphor (36.0%), eucalyptol (13.9%), isoborneol (10.6%), camphene (5.7%) and linalool (4.7%) were the major monoterpenes, while curdione (6.9%), furanogermenone (3.3%) and germacrone (2.8%) were the major sesquiterpenoids. As observed in the above studies, the volatile oils obtained from the rhizomes grown at different geographic regions and climatic conditions, showed considerable difference in their pattern of chemical composition (Usman et al., 2009). The chemical composition of the rhizome oil depends on the genotype, field conditions, and post harvest processing of the rhizomes (Cousins et al., 2007).

### 3.3. Antioxidant activity

Oxidation of biological molecules induces a variety of pathological events such as atherogensis, carcinogenesis and aging (Finkel and Holbrook, 2000). These damages are caused due to the presence of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Therefore the concept of pharmacological supplements to defend against ROS/RNS with antioxidants has become an intense area of research (Adhikari et al., 2007). Thus, supplementation of antioxidants and phytochemicals has attracted considerable attention due to their functional activity. Hence, the measurement of antioxidant potential of the functional food and their active components has become important. The antioxidant activity of volatile oils cannot be attributed only to the presence of phenolic constituents; terpenic alcohols, ketones, aldehydes, hydrocarbons and ethers also contribute to the free radical scavenging activity of some volatile oils (Edris, 2007).

#### 3.3.1. Radical scavenging potentials of volatile oils

The ABTS radical scavenging method is based on the reduction of pre-formed radical cation $ABTS^{\bullet+}$ by the addition of antioxidant. The extent of decolorization of the $ABTS^{\bullet+}$ chromopore measured spectrophotometrically at 734 nm gives the measure of the antioxidant activity of the sample (Re et al., 1999). The extent of inhibition of $ABTS^{\bullet+}$ was plotted as a function of concentration and calculated relative to the reactivity of trolox as a standard, under the same conditions. The result was expressed as the trolox equivalent antioxidant capacity (TEAC). The TEAC values (Fig. 1) were 38.9, 68.0 and 66.9 μM at 1 mg/ml of oil for fresh, dried and cured rhizomes respectively and differ significantly ($p \leq 0.05$). The turmeric oil extracted from dried and cured rhizomes shows higher activity than that of fresh rhizome. This may be attributed to the higher concentration of *ar*-turmerone in the volatile oil of dried rhizome (30.3%) as well as cured (28.3%) than that of fresh (21.0%) rhizome (Table 1). The $IC_{50}$ values for inhibition of ABTS radical cation were 3.3, 1.9 and 2.1 mg/ml for fresh, dried and cured rhizome oils, respectively. This is an excellent method for determining the antioxidant activity of a broad diversity of substances, such as hydrogen-donating antioxidants or scavengers of aqueous phase radicals and of chain-breaking antioxidants or scavengers of lipid peroxylradicals (Re et al., 1999).

The DPPH radical-scavenging activities of the turmeric oils extracted from fresh, dried and cured rhizomes are shown in Fig. 2 and differ significantly ($p \leq 0.05$). The volatile oils notably reduced the concentration of DPPH free radical. The radical scavenging

**Table 1**
Chemical components of essential oil identified from fresh, dried and cured turmeric rhizomes using GC–MS.

| Compound identified[a] | RI[b] | Composition (%) | | | Identification[c] |
|---|---|---|---|---|---|
| | | Fresh | Dried | Cured | |
| α-Pinene | 929 | 0.1 | – | – | RI, MS, CI |
| Myrcene | 984 | 0.1 | – | – | RI, MS, CI |
| α-Phellandrene | 994 | 2.0 | tr | 1.2 | RI, MS |
| δ-2-Carene | 1000 | tr | – | – | RI, MS |
| α-Teripinene | 1006 | tr | – | – | RI, MS |
| *p*-Cymene | 1009 | 0.6 | – | – | RI, MS, CI |
| Eucalyptol | 1017 | 0.8 | tr | 0.4 | RI, MS, CI |
| Limonene | 1019 | 0.1 | – | 0.1 | RI, MS, CI |
| β-Phellandrene | 1049 | 0.1 | – | tr | RI, MS |
| Terpinolene | 1077 | 0.2 | – | tr | RI, MS |
| α-Terpineol | 1170 | tr | tr | 0.1 | RI, MS, CI |
| Thymol | 1276 | tr | tr | – | RI, MS, CI |
| β-Caryophyllene | 1400 | 0.5 | 0.2 | 0.4 | RI, MS |
| (Z,Z)-α-farnesene | 1446 | tr | – | – | RI, MS |
| β-Farnesene | 1509 | 0.1 | tr | 0.1 | RI, MS |
| *ar*-Curcumene | 1465 | 1.9 | 1.2 | 1.8 | RI, MS |
| (Z,E)-α-farnesene | 1469 | tr | – | – | RI, MS |
| α-Zingiberene | 1482 | 2.6 | 2.2 | 2.4 | RI, MS |
| α-Bisabolene | 1494 | 0.4 | 1.5 | 0.4 | RI, MS |
| β-Sesquiphellandrene | 1507 | 2.4 | 1.8 | 2.1 | RI, MS |
| Trans-γ-bisabolene | 1515 | 0.2 | 0.1 | – | RI, MS |
| α-Bisabolol | 1533 | 0.1 | – | – | RI, MS |
| *ar*-Turmerol | 1564 | 0.4 | 0.3 | 0.4 | RI, MS |
| Bergamotol, Z-alpha, -trans- | 1581 | 0.1 | – | 0.1 | RI, MS |
| Cedrene | 1590 | 0.3 | – | – | RI, MS |
| *ar*-Turmerone | 1631 | 21.0 | 30.3 | 28.3 | RI, MS |
| α-Turmerone | 1664 | 33.5 | 26.5 | 24.8 | RI, MS |
| β-Turmerone | 1677 | 18.9 | 19.1 | 21.1 | RI, MS |

[a] Compound listed in the order of elution from a Elite-1 column.
[b] Retention indices relative to $C_8$–$C_{26}$ *n*-alkanes on the Elite-1 column.
[c] RI: identification based on retention index, MS: identification based on comparison of mass spectra, CI: co-injection with authentic standard reference material. tr < 0.1%.

**Table 2**
Absolute quantities[1] of chemical components of essential oil extracted from fresh, dried and cured turmeric rhizomes using GC.

| Compound identified | Composition (mg/100 g) | | |
|---|---|---|---|
| | Fresh | Dried | Cured |
| α-Pinene | 2.8 ± 0.2 | – | – |
| Myrcene | 2.8 ± 0.3 | – | – |
| α-Phellandrene | 55.5 ± 3.8[a] | tr | 42.1 ± 2.8[b] |
| δ-2-Carene | tr | – | – |
| α-Teripinene | tr | – | – |
| *p*-Cymene | 16.6 ± 0.5 | – | – |
| Eucalyptol | 22.2 ± 0.9[a] | tr | 14.0 ± 0.7[b] |
| Limonene | 2.8 ± 0.1[a] | – | 3.5 ± 0.4[b] |
| β-Phellandrene | 2.2 ± 0.2[a] | – | 1.0 ± 0.1[b] |
| Terpinolene | 5.5 ± 0.3 | – | tr |
| α-Terpineol | tr | tr | tr |
| Thymol | tr | tr | – |
| β-Caryophyllene | 13.9 ± 0.2[a] | 3.9 ± 0.2[b] | 14.0 ± 1.2[a] |
| (Z, Z)-α-Farnesene | tr | – | – |
| β-Farnesene | 2.5 ± 0.2[a] | tr | 2.8 ± 0.3[a] |
| *ar*-Curcumene | 52.7 ± 2.4[a] | 28.8 ± 0.2[b] | 63.1 ±5.2[c] |
| (Z,E)-α-farnesene | tr | – | – |
| α-Zingiberene | 72.1 ± 0.7[a] | 52.9 ± 2.2[b] | 84.2 ± 9.5 [c] |
| α-Bisabolene | 12.2 ± 3.6[a] | 36.6 ± 0.3[b] | 14.3 ± 0.7[c] |
| β-Sesquiphellandrene | 66.6 ± 3.4 [a] | 43.3 ± 0.8[b] | 73.7 ± 7.7[c] |
| Trans-γ-bisabolene | 4.2 ± 0.2[a] | 2.1 ± 0.1[b] | – |
| α-Bisabolol | 3.1 ± 0.1 | – | – |
| *ar*-Turmerol | 11.1 ± 0.3[a] | 7.2 ± 1.5[b] | 14.0 ± 0.8[c] |
| Bergamotol, Z-alpha, -trans- | 1.4 ± 0.2[a] | – | 2.1 ± 0.1[b] |
| Cedrene | 7.5 ± 0.3 | – | – |
| *ar*-Turmerone | 582.5 ± 22.5[a] | 728.3 ± 22.7[b] | 992.4 ± 17.2[c] |
| α-Turmerone | 929.2 ± 13.2[a] | 636.9 ± 27.2[b] | 869.6 ± 22.9[c] |
| β-Turmerone | 524.3 ± 29.7[a] | 459.1 ± 25.3[b] | 739.9 ± 20.7[c] |
| Grouped compounds (%) | | | |
| Monoterpene hydrocarbon | 3.2 | 0.03 | 1.4 |
| Oxygenated monoterpenoids | 0.8 | – | 0.5 |
| Sesquiterpene | 8.9 | 7.3 | 7.7 |
| Oxygenated sesquiterpenoids | 73.4 | 75.9 | 74.2 |

[1] Values are mean ± SD of three independent experiments.
Data points with different superscript within the same rows differ significantly at $p \leq 0.05$ according to DMRT.

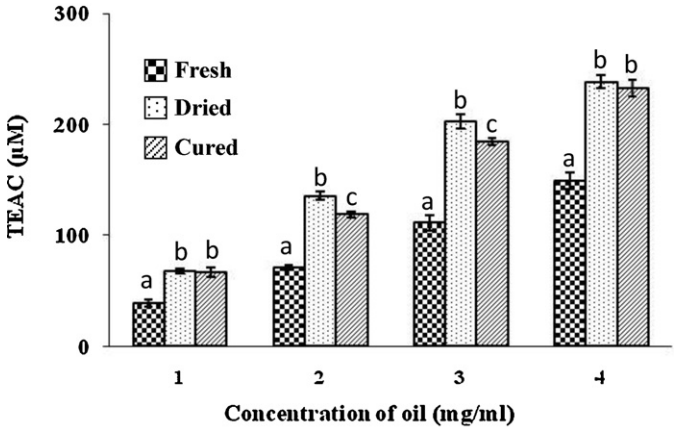


**Fig. 1.** Comparison of trolox equivalents antioxidant capacity (TEAC-µM) of turmeric oil from fresh, dried and cured rhizome using ABTS method. Data points with different superscript within the same concentration differ significantly at $p \leq 0.05$.

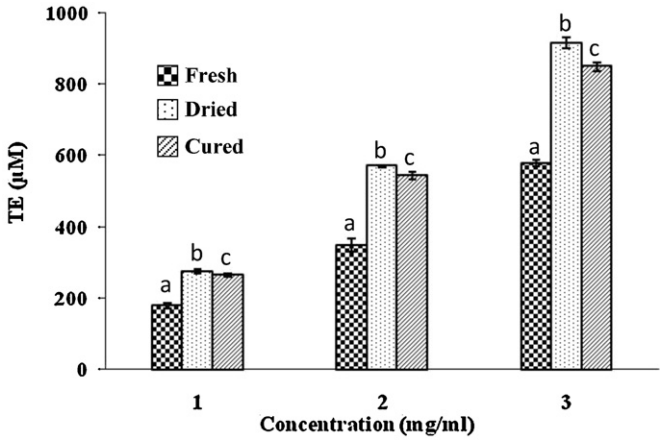


**Fig. 3.** Ferric reducing antioxidant potential (trolox equivalents-µM) of turmeric oil from fresh, dried and cured rhizomes. Data points with different superscript within the same concentration differ significantly at $p \leq 0.05$.

potential of turmeric oil was much lower when compared to that of standard BHA and trolox. Standard BHA and trolox showed a high percentage of radical scavenging activity of 60–90% at 5–50 µg/ml levels, whereas turmeric oil sample showed the same scavenging activity at higher concentrations (4–5 mg/ml). The $IC_{50}$ values were 4.5, 3.5 and 3.9 mg of oil from fresh, dried and cured rhizomes, respectively to quench DPPH free radicals. The antioxidant potential of dried and cured rhizome oils was higher than that of fresh rhizome oil due to the higher concentration of *ar*-turmerone (30.3 and 28.3%, Table 1) in the dried and cured rhizome oils respectively. The order of inhibition of the DPPH radical is dried > cured > fresh rhizome oil. Antioxidant capacity of curcumin-free turmeric oil was evaluated by DPPH radical scavenging assay and was showed to possess appreciable radical scavenging capacity (Yu et al., 2008). This result compliments our findings. Sacchetti et al. (2005) had reported similar radical scavenging activity for turmeric volatile oil. The results obtained show that volatile oils may be considered as potential natural antioxidants which can be formulated as a part of daily supplements or additives to prevent oxidative stress that contributes to many degenerative diseases (Edris, 2007).

### 3.3.2. Ferric reducing antioxidant potential (FRAP) assay

In this assay, at low pH ferric tripyridyltriazine ($Fe^{III}$-TPTZ) complex is reduced to ferrous [Fe(II)] form, an intense blue colored complex with absorption maximum at 593 nm by the electron donating action of antioxidant (Benzie and Strain, 1996). The ferric reducing potential of the volatile oils is shown in Fig. 3 and differs significantly ($p \leq 0.05$). Increase in the concentration of oil increases the absorbance value indicating the formation of Fe(II)/TPTZ from colorless oxidized Fe(III) form. The reducing ability of the volatile oil was found to be in the order fresh < cured < dried. The volatile oil extracted from dried and cured rhizomes shows higher reducing activity than that of fresh rhizome. The TEAC values of FRAP assay were 178.4, 276.8 and 264.1 µM at 1 mg/ml level of the volatile oil obtained from fresh, dried and cured rhizomes, respectively. It is clear from the obtained results that the antioxidant compounds are capable of scavenging free radicals and reducing oxidants (ferric ion) (Li et al., 2008).

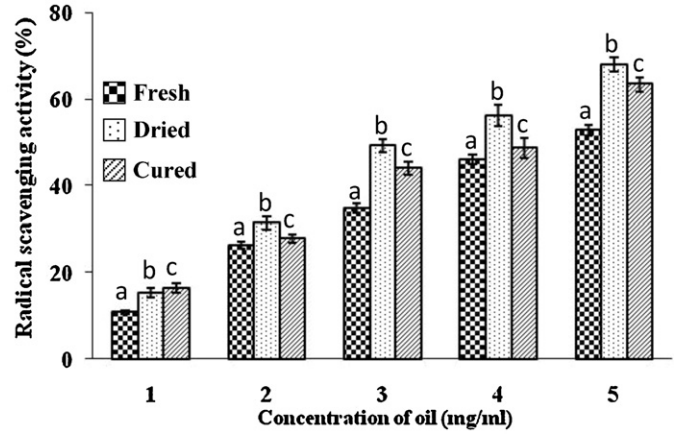


**Fig. 2.** Radical scavenging activity (%) of turmeric oil from fresh, dried and cured rhizomes using DPPH method. Data points with different superscript within the same concentration differ significantly at $p \leq 0.05$.

### 3.3.3. Total antioxidant capacity by phosphomolybdenum assay

The total antioxidant capacity assay is based on the reduction of Mo(V) to Mo(IV) by the antioxidant substance in the sample and subsequent formation of a green phosphate/Mo(V) complex at acidic pH with an absorbance maximum at 695 nm (Prieto et al., 1999). The total antioxidant capacity of volatile oil increases with the increase in the concentration of the sample. The total antioxidant capacity of the volatile oil was found to be in the order dried > cured > fresh. Fresh, dried and cured rhizome oils showed antioxidant capacity of 358, 686 and 638 mM of ascorbic acid equivalents per 1 mg of oil respectively. The volatile oil extracted from dried and cured samples was found to be similar up to a concentration of 100 µg/ml (Fig. 4). The volatile oil from dried rhizomes showed higher activity than that of cured rhizome above this concentration. The higher activity oil may be attributed to the higher concentration of *ar*-turmerone (Jayaprakasha et al., 2002).

### 3.3.4. Reducing power assay

In this assay the ability of the sample to reduce Fe(III) to Fe(II) was determined and compared with that of ascorbic acid. The volatile oil samples extracted from the fresh, dried and cured rhizomes showed reducing potential in a concentration-dependent manner. The reducing power increased with increase in the sample concentration (Fig. 5). The reducing power of turmeric oil was much lower when compared to that of standard ascorbic acid. Standard ascorbic acid (10–100 µg/ml), which is a well-known reducing agent showed maximum reducing value (absorbance at 700 nm is 0.8) at 100 µg/ml. The volatile oil samples followed the same trend as that for the other antioxidant assays. The oil extracted from dried and cured rhizomes showed higher reducing ability (absorbance of

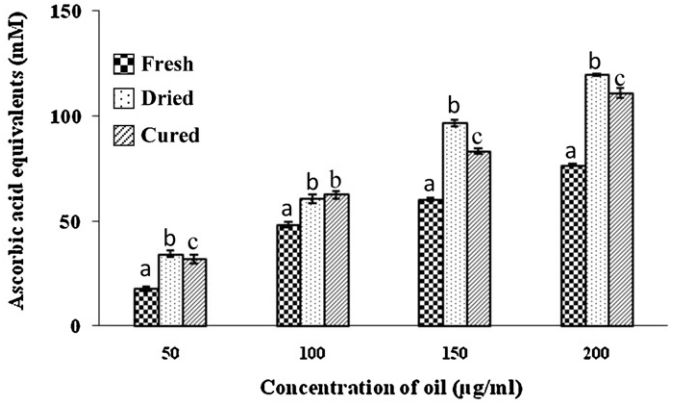


**Fig. 4.** Total antioxidant capacity (ascorbic acid equivalents-mM) of turmeric oil extracted from fresh, dried and cured rhizomes using phosphomolybdenum method. Data points with different superscript within the same concentration differ significantly at $p \leq 0.05$.

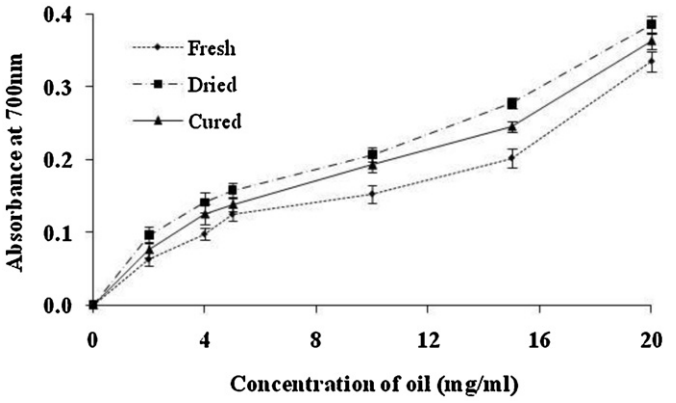


**Fig. 5.** Reducing power of turmeric oil at different concentrations from fresh, dried and cured rhizomes.

0.4 at 700 nm) than oil from fresh rhizome. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Duh, 1998).

## 4. Conclusion

In present days, phytochemicals with functional properties are in great demand in foods, cosmetics and pharmaceuticals sectors. Thus there is a need to conduct studies on chemical composition of volatile oil and to associate these with the functional properties. In this regard, the volatile oil from fresh, dried and cured turmeric rhizomes was isolated and characterized chemically and its functionality with respect to its antioxidant potential was studied using complementary assay methods. High yield of oil was obtained from cured rhizome when compared with that of fresh and dried rhizomes. The volatile oil extracted from dried as well as cured rhizomes showed a higher antioxidant potential than that of fresh rhizome oil. Hence it may be concluded that the turmeric oil from dried as well as cured rhizome has high antioxidant potential with lower $IC_{50}$ values. The turmeric oil with appreciably high antioxidant potential can be used as a functional food and may find application in food industry as a possible alternative to synthetic antioxidants.

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