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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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ABSTRACT

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

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