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Chemical composition and antioxidant activities of essential oil and oleoresins from *Curcuma zedoaria* rhizomes, part-74

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ABSTRACT

The chemical composition of essential oil and oleoresins (ethanol, ethyl acetate and isopropanol) from rhizomes of *Curcuma zedoaria* was analyzed by the Gas Chromatography–Mass Spectroscopy technique. The antioxidant studies were carried out by peroxide and thiobarbituric acid values, ferric thiocyanate, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, metal chelating and reducing power methods. Curzerenone (31.6%) was the major component in the volatile oil, followed by germacrone (10.8%). In ethanol, isopropanol and ethyl acetate oleoresin, a total of 26, 25, and 40 components were identified respectively and the major ones are curzerenone, germacrone, camphor and curcumenol. Essential oil, oleoresins (ethyl acetate and isopropanol) showed potent antioxidant activity in all the experiments. However, the activity of ethanol oleoresin was found to be lower as compared to ethyl acetate and isopropanol oleoresin.

1. Introduction

It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, heart disease, cancer, ageing, diabetes mellitus and others (Young & Woodside, 2001). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Currently, there is a growing interest toward natural antioxidants of herbal resources (Velioglu, Mazza, Gao, & Oomah, 1998). Epidemiological and *in-vitro* studies on spices, medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity of exerting protective effects against oxidative stress in biological system. *C. zedoaria* (Berg.) rosc, (Zingiberaceae) has long been used as folk medicine.

Curcuma (Zingiberaceae) is a large genus of rhizomatous herbs distributed in tropical and subtropical regions especially in India, Thailand, Malay Archipelago, IndoChina, and Northern Australia. Many phytochemical studies on the extracts and essential oils of several *Curcuma* species, especially *C. longa*, have identified curcuminoids and sesquiterpenoids as the major components (Itokawa, Shi, Akiyama, Morris-Natschke, & Lee, 2008), and these compounds have been identified as the major groups of antioxidants in the plants. The antioxidant activity of *Curcuma longa*, has been measured by various chemical methods such as DPPH radical scavenging activity assay, superoxide anion radical scavenging activity assay,

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ferric reducing/antioxidant power (FRAP) assay, and metal chelating activity assay (Zhao, Zhang, Yang, Lv, & Li, 2010; Himaja, Ranjitha, Ramana, Anand, & Karigar, 2010).

The objectives of the present study were to investigate the antioxidant activity of the essential oil and oleoresins of *C. zedoaria*, compared to synthetic antioxidants carrying out *in-vitro* tests including determination of peroxide, thiobarbituric acid values; scavenging effects on 1,1′diphenyl-2-picrylhydrazyl (DPPH); reducing power and total antioxidant activity by ferric thiocyanate (FTC) methods. The chemistry of volatile oil and oleoresins has also been investigated by GC–MS as the literature available is very scant.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used were of analytical grade. Thiobarbituric acid (TBA), DPPH and linoleic acid are of Acros (New Jersey, USA), butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and propyl gallate (PG) from SD Fine Chemicals Ltd. Mumbai; tween 20 and ferrozine from Merck Pvt. Ltd. Mumbai. The mustard oil was purchased from local oil mill, Gorakhpur, India.

2.2. Extraction of essential oil and oleoresins

The dry rhizomes of *C. zedoaria* were purchased from the local market of Gorakhpur, India and voucher specimens (Specimen no. 138) were deposited at the Herbarium of the Science Faculty of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur, India. The rhizomes were washed, sun dried and pulverized into a fine powder. The essential oil was extracted from spice powder by the hydrodistillation method using a Clevenger's type apparatus while oleoresins were extracted by Soxhlet's apparatus using different organic solvents (ethanol, ethyl acetate and isopropyl alcohol).

2.3. Chemical analysis

2.3.1. Sample preparation and GC–MS analysis

About 10 mg of viscous ethanol (EtOH) oleoresin was dissolved with 220 μl of dry pyridine and 80 μl of bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added. The reaction mixture was sealed and heated for 30 min at 60 °C to obtain trimethylsilyl derivatives (TMS) (Isidorov, Kotowska, & Vinogorova, 2005). These derivatives not only have improved stability against hydrolysis, but they also have the added advantage of distinctive fragmentation patterns, which makes them useful in a variety of GC/MS applications (Kühnel et al., 2007; Blau & King, 1997). Essential oil, oleoresins and TMS were analyzed by GC–MS on a HP 6890 gas chromatograph with mass selective detector MSD 5973 (Agilent Technologies, USA). This device was fitted with a HP-5 ms (5% phenylmethyl siloxane) fused silica column (30 m × 0.25 mm × 0.25 μm), with electronic pressure control (EPC) and split/splitless injector. Helium flow rate through the column was 1 ml/min in constant flow mode. Electron impact mass spectrometry (EIMS) spectra were obtained at 70 eV of ionization energy, at the temperature of the source 220 °C and that of quadrupole 150 °C. The MSD was set to scan 41–750 amu. The oven temperature program was: 40–310 °C at 3 °C/min, where it was held for 20 min. The values of retention times of *n*-alkanes (C_9-C_{28}, C_{30}, C_{32}, C_{36}, C_{38} and C_{40}) and separated compounds were used to calculate retention indices (RI^{Exp}). Components were identified with the aid of an automatic system of processing data of GC–MS supplied by NIST mass spectra library.

2.4. Antioxidant properties

2.4.1. Lipid peroxidation assays

2.4.1.1. *Sample preparation*. The unrefined crude mustard oil samples (50 g each) containing 200 ppm (w/v) concentrations of essential oil or oleoresins, BHA, BHT and PG were prepared. Mustard oil without any additive was used as a control sample. All the samples were exposed to accelerated oxidation by incubating at 70 °C in darkness.

2.4.1.2. *Peroxide value (PV)*. Total peroxide and hydroperoxide oxygen content of the mustard oil samples were determined by measuring PV at regular intervals according to the procedure as reported by Hortwitz (2002). A 5 g of mustard oil sample was dissolved in 30 ml of glacial acetic acid–chloroform (3:2) and mixed with 0.5 ml of saturated potassium iodide solution. After 1 min, 30 ml distilled water was added and the whole solution was titrated against 0.01 N sodium thiosulphate using starch as an indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. The PV in mill equivalent per kilogram (meq/Kg) of sample was calculated as

$$\text{Meq of peroxide/kg of oil} = \frac{S \times N \times 1000}{\text{Weight of oil}}$$

where *S* is volume of sodium thiosulphate consumed and *N* is normality of sodium thiosulphate solution.

2.4.1.3. *TBA value*. TBA value of different samples was determined according to the method previously reported by Pokorny and Dieffenbacher (1989). About 100 mg of oil sample was dissolved in 25 ml of 1-butanol. A 25 ml aliquot of the above solution was mixed thoroughly with 5.0 ml of TBA reagent (200 mg TBA in 100 ml 1-butanol) and incubated at 95 °C. After 2 h, the reaction mixture was cooled to room temperature under running water and absorbance was measured at 530 nm with spectrophotometer (Aimil Spectrochem NV201). At the same time, a reagent blank (without TBA reagent) was also done. The thiobarbituric acid value (meq of malonaldehyde/g) was calculated as

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

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

2.4.2. Individual complementary assays

2.4.2.1. *DPPH free radical scavenging activity*. The capacity of essential oil and oleoresins to scavenge the DPPH radical was determined by the method described by Cuendet, Hostettmann, Pottera, and Dyatmilko (1997). DPPH radical absorbs at 517 nm and the antioxidant activity can be determined by monitoring