

# Evaluation of Antioxidant Activities and Antimutagenicity of Turmeric Oil: A Byproduct from Curcumin Production

Guddadarangavvanahally K. Jayaprakasha, Bhabani S. Jena, Pradeep S. Negi and Kunnumpurath K. Sakariah\*

Human Resource Development, Central Food Technological Research Institute, Mysore 570013, India. Fax: 0821-516308. E-mail: gkjp@yahoo.com

\* Author for correspondence and reprints requests

Z. Naturforsch. **57c**, 828–835 (2002); received April 30/June 7, 2002

Turmeric Oil, Antioxidant Activity, Antimutagenicity

Curcumin removed turmeric oleoresin (CRTO) was extracted with hexane and concentrated to get turmeric oil, and that was fractionated using silica gel column chromatography to obtain three fractions. These fractions were analyzed by GC and GC-MS. Turmeric oil contained aromatic turmerone (31.32%), turmerone (15.08%) and curlone (9.7%), whereas fractions III has aromatic turmerone (44.5%), curlone (19.22%) and turmerone (10.88%) as major compounds. Also, oxygenated compounds (5,6,8–10) were enriched in fraction III. Turmeric oil and its fractions were tested for antioxidant activity using the  $\beta$ -carotene-linoleate model system and the phosphomolybdenum method. The fraction III showed maximum antioxidant capacity. These fractions were also used to determine their protective effect against the mutagenicity of sodium azide by means of the Ames test. All the fractions and turmeric oil exhibited a markedly antimutagenicity but fraction III was the most effective. The antioxidant effects of turmeric oil and its fractions may provide an explanation for their antimutagenic action.

## Introduction

The rhizome of turmeric (*Curcuma longa* L.) has a rich history in India as spice, food preservative, and coloring agent and has been used for centuries in the Ayurvedic system of medicine. Long before the time of cheaper synthetic food preservatives and colouring agents, spices like turmeric played a key role as food additive (Majeed *et al.*, 1995). Its use as a remedy for hypercholesterolemia, arthritis, indigestion and liver problem has been known since long (Srimal, 1997). The continuing research indicates that turmeric and its active principle curcumin have unique antioxidant, antimutagenic, antitumorigenic, and anticarcinogenic, antiinflammatory, antiarthritic, antimicrobial, and hypocholesterolemic properties as reviewed elsewhere (Majeed *et al.*, 1995; Miquel *et al.*, 2002).

Aromatic turmerone (20–30%) was reported to be the major compound present in turmeric volatile oil (Govindarajan, 1980), which is a mosquito repellent (Tawastin *et al.*, 2001) and may be an effective drug for the treatment of respiratory disease (Li *et al.*, 1998) and dermatophytosis (Apisariyakul *et al.*, 1995). Synthetic turmerone appears to act as anticarcinogenic (Baik *et al.*, 1993). Anti-

venom activity of turmerone isolated from turmeric has also been reported (Ferreira *et al.*, 1992). Recently, turmeric oil isolated from CRTO was found to be both antifungal (Jayaparakasha *et al.*, 2001) and antibacterial (Negi *et al.*, 1999).

Curcumin, the yellow coloring pigment of turmeric is industrially produced using turmeric oleoresin as the source material. The mother liquor (approximately 70–80%) after isolation of curcumin from oleoresin is known as curcumin removed turmeric oleoresin (CRTO). It is composed of oil, resin, and non-extractable curcumin and has no commercial value at present (Saju *et al.*, 1998). In the present study we report the isolation, fractionation and identification of turmeric oil and its column fractions from CRTO, and their antioxidant activity and antimutagenicity.

## Materials and Methods

### Materials

All solvents and other chemicals used were of analytical grade and obtained from Merck, Mumbai, India. Professor B. N. Ames, University of Berkeley, California kindly supplied *Salmonella*

*typhimurium* strain, TA-100. Curcumin removed turmeric oleoresin was obtained from M/S Flavour and Essences (P) Ltd., Mysore.

#### *Extraction of turmeric oil*

Twenty grams of CRTO were stirred with 50 ml of hexane for 15 min and the extract was filtered. The residue was extracted twice with 50 ml hexane and filtered. The extracts were combined and the solvent was removed under vacuum (Büchi, Switzerland) with a yield of 11.8 ml (v/w) of turmeric oil.

#### *Fractionation of turmeric oil*

Seven grams of turmeric oil was impregnated with 10 g of silica gel and loaded on to a silica gel column. The column was eluted successively with 1500 ml each of hexane, hexane:benzene (1:1 v/v) and benzene. The solvents from the elutes were evaporated under vacuum to get three fractions, the yields of which were 21, 42.2 and 33.1% respectively.

#### *GC analysis*

Turmeric oil and its column fractions were analyzed using a Shimadzu GC 15A Chromatography equipped with a FID detector, using SE-30 column (3.0 m × 1/8"). Oven temperature was programmed from 60° C for 3 min to 225° C at the rate of 3° C/min at which temperature of the column was maintained for 3 min; injector port temperature was 225° C; detector temperature was 250° C; nitrogen as carrier gas was 40 ml/min. Peak areas were computed by a Shimadzu C-R4A chromatopac data processor.

#### *GC-MS analysis*

The turmeric oil and its column fractions were analyzed using a Shimadzu 17A-GC chromatograph equipped with a QP-5000 (Quadrupole) Mass Spectrometer. The sample was diluted 25 times with chloroform and 1 µl was injected. A fused silica column SPB<sup>TM-1</sup> (30 m × 0.32 mm film thickness 0.25 µm) coated with polydimethylsiloxane was used. Injector port temperature was 225° C; detector temperature was 250° C and oven temperature was maintained at 60° C for 3 min and then increased to 225° C at the rate of 2° C/min at which temperature of the column was

maintained for 5 min; helium was the carrier gas at a flow rate of 1 ml/min; split ratio was 1:25; ionization voltage, 70 eV.

#### *HPLC analysis of curcuminoids*

The curcuminoids in the turmeric oil and its fractions were analyzed by HPLC method (Jayaprakasha *et al.*, 2002). The high-performance liquid chromatographic system consisted of a Hewlett Packard Quaternary HPLC model HP 1100 Series (Hewlett-Packard, CA, USA), fitted with a Waters µ-Bondapack<sup>TM</sup> (Waters Corporation, Milford, MA, USA) C<sub>18</sub> column (300 × 4.6 mm I.D). The injection system (Rheodyne) used was a 20 µl sample loop. A HP 1100 Series Variable Wavelength Detector used at wavelength of 425 nm was used for detection. The elution was carried out with gradient solvent systems with a flow rate of 1.0 ml/min at ambient temperature. The mobile phase consisted of methanol (A), 2% acetic acid (B) and acetonitrile (C). Quantitative levels of curcuminoids were determined using above solvents programmed linearly from 45% to 65% acetonitrile in B for 0 to 15 min. Then 65% to 45% acetonitrile in B for 15–20 min, with a constant of 5% A. The compounds were quantified using the HP ChemStations software. The turmeric oil and its fractions were diluted with acetone (1:10) and 20 µl injected into HPLC. Curcuminoids concentrations were calculated based on linear calibration functions and with regard to the dilution factor. The content of curcuminoids was expressed as g/100 g oil.

#### *Antioxidant assay by β-carotene-linoleate model system*

The antioxidant activity of turmeric oil and its column fractions were evaluated by the β-carotene-linoleate model (Jayaprakasha and Jaganmohan Rao, 2000). 0.2 mg of the β-carotene in 0.5 ml of chloroform, 20 mg of linoleic acid and 200 mg of Tween-40 (polyoxyethylene sorbitan mono-palmitate) were mixed together. The chloroform was removed at 40° C under vacuum using a rotary evaporator. The resulting solution was immediately diluted with 10 ml of triple-distilled water and the emulsion was mixed well for 1 min. The emulsion was further diluted with 40 ml of oxygenated water before use. 4 ml aliquots of this mixture

were transferred into different tubes containing 0.2 ml of samples at 100 µg/ml concentrations in ethanol, butylated hydroxyanisole (BHA) was used for comparative purposes. A control containing 0.2 ml of ethanol and 4 ml of the above mixture was prepared. Optical density (OD) at 470 nm were taken for the all extracts and pure compounds immediately ( $t = 0$ ) at 15 min intervals for 1.5 h ( $t = 90$ ). The tubes were incubated at 50° C in a water bath. All determinations were performed in triplicate. Measurement of OD was continued until the colour of β-carotene disappeared in the control. The antioxidant activities (AA) of the samples were evaluated in terms of bleaching the β-carotene using the following formula.  $AA = 100[1 - (A_t - A_c) / (A_o - A_c)]$  where  $A_o$  and  $A_c$  are the OD measured at zero time of the incubation for test sample and control, respectively.  $A_t$  and  $A_c$  are the OD measured in the test sample and control, respectively, after incubation for 90 min.

#### *Evaluation of antioxidant capacity by phosphomolybdenum method*

The total antioxidant capacity of turmeric oil, and its different fractions was evaluated by the method of Prieto *et al.*, (1999). An aliquot of 0.1 ml of sample (100 µg) solution was combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95° C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in Genesys-5-UV spectrophotometer (Milton Roy, New York). A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. For samples of unknown composition, fat soluble antioxidant capacity was expressed as equivalents of α-tocopherol (mmol/g of turmeric oil or its fraction) (Prieto *et al.*, 1999).

#### *Antimutagenicity assay*

The standard plate incorporation test and other procedures were carried out according to Maron and Ames (1983). In the antimutagenicity test, the

inhibitions of mutagenic activity of the sodium azide by the test samples were determined. The turmeric oil and its column fractions (625, 1250 and 2500 µg/plate) were assayed by plating with molten soft agar (2 ml) containing 10 h old culture (0.1 ml) of strain of *S. typhimurium* TA100. Positive and negative controls were also included in each assay. Sodium azide was used as a diagnostic mutagen (1.5 µg per plate) in positive control and plates without sodium azide and without test samples were considered as negative controls. His<sup>+</sup> revertants were counted after incubation of the plates at 37° C for 48 h. Each sample was assayed using duplicate plates and the data presented here are mean ± SD of three independent assays. The mutagenicity of sodium azide in the absence of test samples was defined as 100% or 0% inhibition. The calculation of % inhibition was done according to the formula given by Ong *et al.* (1986): % inhibition =  $[1 - T/M] \times 100$  where T is number of revertants per plate in presence of mutagen and test sample and M is number of revertants per plate in positive control. The number of spontaneous revertants was subtracted from numerator and denominator.

The antimutagenic effect was considered moderate when the inhibitory effect was 25–40% and strong when more than 40%. Inhibitory effects of less than 25% was considered as weak and was not recognised as positive result (Ikken *et al.*, 1999).

## **Results and Discussion**

It was found that CRTO contains substantial amount of turmeric oil (59% v/w) which is comparable with earlier reports (Jayaprakasha *et al.*, 2001; Saju *et al.*, 1998). Turmeric oil was fractionated using silica gel column chromatography, which yielded 21, 42.2 and 33.1% of fractions I, II and III, respectively. Retention indices for all the compounds were determined using *n*-alkanes as standards (Jennings and Shibamoto, 1980). The compounds were identified by comparison of retention indices with those reported in the literature (Jennings and Shibamoto, 1980; Davis, 1990) and by matching their mass spectral fragmentation patterns with those stored in the spectrometer database, using the NIST62-Lib (Shimadzu Corporation, Japan) MS library or comparison of MS data with those reported in literature (Jennings and

Shibamoto, 1980; Ten Noever Bravw *et al.*, 1988; Adams, 1989). The chemical constituents of turmeric oil, column fractions were determined by GC and GC-MS. Table I shows the retention times, Kovats index and chemical constituents of turmeric oil and its column fractions. It was found that aromatic turmerone and turmerone were the major compounds in turmeric oil and its fractions. Further, some oxygenated compounds (2,3,5,6,8–10) are also identified in turmeric oil and its fractions (Fig. 1). The presence of curcuminoids was analyzed in turmeric oil and its fractions by HPLC method. The curcuminoids were found in turmeric oil to the extent of 0.10–0.15%, but in column fractions the curcuminoids were not observed. This may be due to the silica gel fractions using hexane: benzene as an eluent. In this study we were interested in the antioxidant and antimutagenic activities of turmeric oil and its fractions (devoid of curcuminoids), therefore the column was not eluted with more polar solvents.

The antioxidant activity of turmeric oil and its fractions at 100 µg/ml concentration compared with butylated hydroxyanisole is presented in Fig. 2. It shows the decrease in absorbance of β-carotene in the presence of turmeric oil and its column fractions and BHA with the coupled oxidation of β-carotene and linoleic acid. The addition of turmeric oil and its column fractions and butylated hydroxyanisole at 100 µg/ml concentra-

tions prevents the bleaching of β-carotene to different degrees. Turmeric oil and its fractions I, II, and III showed 65, 43, 46 and 49% antioxidant activity at 100 µg/ml concentration. The marked antioxidant activity of turmeric oil may be due the presence of traces of curcuminoids, which were extracted along with turmeric oil from CRTO. However, the decreasing order of antioxidant activity among the fractions was found to be III > II > I. The antioxidant activity of fractions may be attributed to the synergistic effects of their constituents (Table I).

The quantitative antioxidant capacity of the turmeric oil and its fractions were measured spectrophotometrically through the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex with a maximum absorption at 695 nm. The antioxidant capacity of turmeric oil and its fractions was found to decrease in the order III > crude oil > II > I. However, fraction III and I showed the highest and lowest antioxidant capacities respectively (Fig. 3). The decreasing order of antioxidant capacity of turmeric oil and its fractions is not absolutely correlating with the order of antioxidant activity found in β-carotene-linoleate model system. The phosphomolybdenum method has been used to investigate the total antioxidant capacity of the extracts. On the other hand, the β-

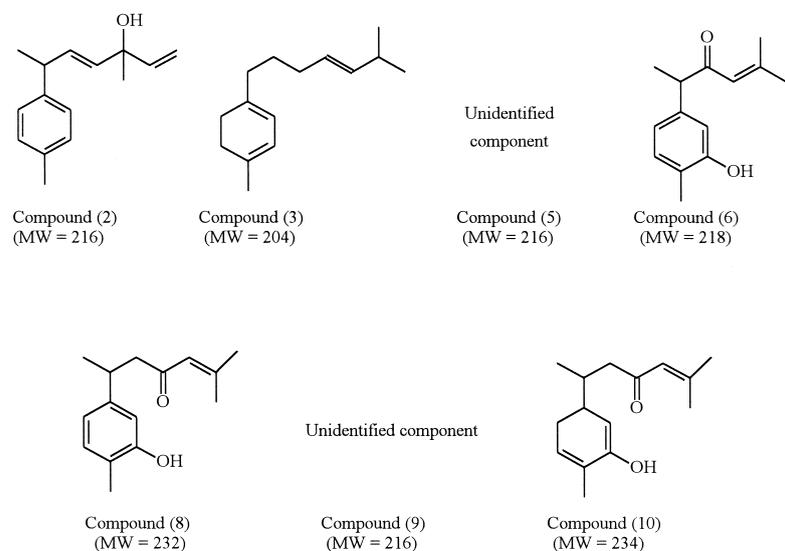


Fig. 1. Structures of compounds identified in turmeric oil and its fractions.



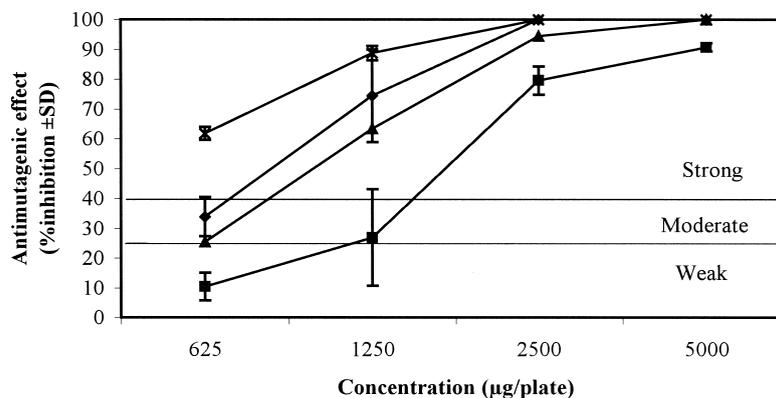


Fig. 4. Inhibitory effect of turmeric oil and its fractions against the mutagenicity of sodium azide to *Salmonella typhimurium* TA 100.

◆ Crude oil. ■ Fraction I. ▲ Fraction II. ✕ Fraction III.

carotene-linoleate model system has been used to analyze the antioxidant activity of the extracts. At this conjecture we can suggest for the *in vivo* investigations of antioxidant activity of turmeric oil and its fractions. The active principle in turmeric is a group of phenolic compounds including curcumin which is very well known for its strong antioxidant activity (Miquel *et al.*, 2002). However, in the present investigation we report that the turmeric oil and its fractions are also effective antioxidants. It seems that the major compounds present in the turmeric oil and its fractions exert either the synergistic or additive actions for their antioxidant activity.

The protective action of turmeric oil and its fractions against the mutagenicity of sodium azide was evaluated by the Ames test using *S. typhimurium* TA100, as presented in Fig. 4. It appears that, the antimutagenicity of turmeric oil and its column fractions increases with doses. Turmeric oil and its fractions showed an antimutagenicity ranging from weak to strong and even 100%, depending on concentration of the test samples. At the concentration of 625 and 1250 µg/plate, the antimutagenicity of crude oil and its fractions was decreased in the order of III > crude oil > II > I. Fraction III and fraction I showed the strongest and weakest antimutagenicity, respectively, against the mutagenicity of sodium azide. Aromatic turmerone and turmerone were found to be the major compounds present in the turmeric oil and its fractions in the decreasing order of III (55.58%) >

crude oil (46.40%) > II (42.98%) > I (22.19%). Synthetic turmerone has been reported to act as neoplasm inhibitor and anticarcinogenic (Baik *et al.*, 1993). The extent of the presence of aromatic turmerone and turmerone in turmeric and its fractions may be responsible for their antimutagenic activity. The active principle in turmeric is the curcumin, which has strong antimutagenic activity both *in vitro* and *in vivo* (Goud *et al.*, 1993). The presence of traces of curcuminoids in crude oil may also be responsible for its antimutagenicity.

It has been reported that mutation induced by numerous mutagens was reduced by active oxygen scavengers (Kim *et al.*, 1991; Ueno *et al.*, 1991). It has also been suggested that compounds, which possess antioxidant activity, can inhibit mutation and cancer because they can scavenge free radical or induce antioxidative enzyme (Hochstein and Atallah, 1988). Goud *et al.*, (1993) observed significant increase of xenobiotic metabolizing enzymes such as UDP glucuronyl transferase and glutathione-S-transferase in the liver of turmeric fed rats. Similarly curcumin an antioxidant, isolated from turmeric was shown to induce glutathione-S-transferase and glutathione peroxidase which are involved in detoxification of electrophilic products of lipid peroxidation and contribute to the anti-inflammatory and anticancer activities (Piper *et al.*, 1998). Azuine and Bhide (1992) found the protective effect of dietary turmeric on BP-induced forestomach and DMBA-induced skin tumors with simultaneous increase of glutathione

level and activity of glutathione-S-transferase in the liver of mice. However, the antioxidant, antimutagenic and other biological activities of curcuminoids have been reported (Majeed *et al.*, 1995; Miquel *et al.*, 2002). In our present study turmeric oil and its fractions are having both antioxidant and antimutagenic activities. Dietary supplementation of antioxidants present in fruits and vegetables are thought to decrease free radical attack on DNA and hence protect against mutation that causes cancer (Duthie *et al.*, 1996). In the present study it was observed that the decreasing order of antioxidant activity of turmeric oil and its fractions is almost equivalent to the decreasing order of their antimutagenicity i.e. III > crude oil > II > I. The antioxidative properties of turmeric oil and its fractions have therefore played an important role with regard to their antimutagenicity, which seem to explain the action of their antimutagenicity. Moreover, it has been observed that aromatic turmerone and turmerone are the major

constituents found in turmeric oil and its fractions, and fraction III was found to contain highest percentage of aromatic turmerone and turmerone. Probably aromatic turmerone alone or in synergy with turmerone, curlone and compounds (5,6,8–10) is responsible for the highest antioxidant and antimutagenic activities in fraction III. Further work is required to assess the process of chemoprevention of mutation and carcinogenesis using turmeric oil with special reference to its fraction III. Thus there will be a value addition to the by-product of curcumin production, which has no commercial application at present.

#### Acknowledgements

We wish to thank Prof. B. N. Ames, University of Berkeley, California, for supplying *Salmonella* strains. We also thank Mr. Shivaswamy, Central Instrumentation Facilities, Central Food Technological Research Institute, for assistance in obtaining the GC-MS analysis.

- Adams R. P. (1989), Identification of Essential Oils by Ion Trap Mass Spectrometry. Academic Press, New York, p. 29.
- Apisariyakul A., Vanittanakom N. and Buddhasukh D. (1995), Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). *J. Ethnopharmacol.* **49**, 163–169.
- Azuine M. A. and Bhide S. V. (1992), Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogenesis in Swiss mice. *Nutrition and Cancer* **17**, 77–83.
- Baik K. U., Jung S. H. and Ahn B. Z. (1993), Recognition of pharmacophore of ar-turmerone for its anticancer activity. *Arch. Pharmacol Res.* **16**, 254–256.
- Davies W. (1990), Gas chromatographic retention indices of Monoterpenes and Sesquiterpenes on methyl Silicone and Carbowax 20 M phases. *J. Chromatogr.* **50**, 1–24.
- Duthie S. J., Ma A., Ross M. A. and Collins A. R. (1996), Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Research* **56**, 1291–1295.
- Ferreira L. A. F., Henriques O. B., Andreoni A. A. S., Vital G. R. F., Campos M. M. C., Habermehl G. G. and DeMoraes V. L. G. (1992), Antivenom and biological effects of ar-turmerone isolated from *Curcuma longa* (Zingiberaceae). *Toxicon* **30**, 1211–1218.
- Goud V. K., Polasa K. and Krishnaswamy K. (1993), Effect of turmeric on xenobiotic metabolizing enzymes. *Plant Foods and Human Nutrition* **44**, 87–92.
- Govindarajan V. S. (1980), Turmeric-Chemistry, Technology and Quality. *CRC Crit. Rev. Food Sci. Nutr.* **12**, 199–301.
- Hiserodt R., Thomas G., Hartman Chi-Tang H. and Robert T. R. (1996), Characterization of powdered turmeric by liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. *J. Chromatogr.* **740**, 51–63.
- Hochstein P. and Atallah A. S. (1988), The nature of oxidant and antioxidant systems in inhibition of mutation and cancer. *Mutat. Res.* **202**, 363–375.
- Ikken Y., Morales P., Martinez A., Marin M. L., Haza A. I. and Cambero M. I. (1999), Antimutagenic effect of fruit and vegetable ethanolic extracts against N-nitrosamines evaluated by the Ames test. *J. Agric. Food Chem.* **47**, 3257–3264.
- Jayaprakasha G. K. and Jaganmohan Rao L. (2000), Phenolic constituents from lichen *Parmotrema stipitum* (Nyl.) Hale and their antioxidant activity. *Z. Naturforsch.* **55c**, 1018–1022.
- Jayaprakasha G. K., Negi P. S., Anandharamakrishnan C. and Sakariah K. K. (2001), Chemical composition of turmeric oil – A byproduct from turmeric oleoresin industry and its inhibitory activity against different fungi. *Z. Naturforsch.* **56c**, 40–44.
- Jayaprakasha G. K., Jaganmohan Rao L. and Sakariah K. K. (2002), An improved HPLC method for the determination of curcumin, demethoxycurcumin and bisdemethoxycurcumin, *J. Agric. Food Chem.* **50**, 3668–3672.

- Jennings W. and Shibamoto T. (1980), Qualitative Analysis of Flavor and Fragrance Volatile by Glass Capillary Gas Chromatography. Academic Press, New York, p. 29.
- Kim S. B., Kim I. S., Yeum D. M. and Park Y. H. (1991), Mutagenicity of Maillard reaction products from D-glucose-amino acid mixtures and possible roles of active oxygens in mutagenicity. *Mutat. Res.* **254**, 65–69.
- Li C., Li L., Luo J. and Huang N. (1998), Effect of turmeric volatile oil on respiratory tract. *Zhongguo Zhong Yao Za Zhi*, **23**, 624–625.
- Majeed M., Badmaev V., Shivakumar U. and Rajendran R. (1995), Curcuminoids- Antioxidant Phytonutrients. Nutriscience Publishers, Inc., Piscataway, New Jersey.
- Maron D. M. and Ames B. N. (1983), Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **113**, 173–215.
- Miquel J., Bernd A., Sempere J. M. and Diaz-Alperi R. A. (2002), The curcuma antioxidants: pharmacological effects and prospects future clinical use. A review. *Arch. Gerontol. Geriatr.* **34**, 37–46.
- Negi P. S., Jayaprakasha G. K., Jaganmohan Rao L. and Sakariah K. K. (1999), Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *J. Agric. Food Chem.* **47**, 4297–4300.
- Ong T., Wong W. Z., Stewart J. D. and Brockman H. E. (1986), Chlorophyllin: a potent antimutagen against environmental and dietary complex mixture. *Mutat. Res.* **173**, 111–115.
- Piper J. T., Sighal S. S., Salameh M. S., Tormen R. T., Awasthi Y. C. and Awasthi, S. (1998), Mechanisms of anticarcinogenic properties of curcumin: the effect of curcumin on glutathione linked detoxification enzymes in rat liver. *Int. J. Biochem. Cell Biol.* **30**, 445–456.
- Prieto P., Pineda M. and Aguilar M. (1999), Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **269**, 337–341.
- Saju K. A., Venugopal M. N. and Mathew M. J. (1998), Antifungal and insect-repellent activities of essential oil of turmeric (*Curcuma longa* L.). *Current Science.* **75**, 660–662
- Srimal R. C. (1997), Turmeric: a brief review of medicinal properties. *Fitoterapia* **LXVIII**, 483–493.
- Tawastin A., Wratten S. D., Scott R. R., Thavara U. and Techadamrongsin Y. (2001), Repellency of volatile oils from plants against three mosquito vectors. *J. Vector Ecol.* **26**, 76–82.
- Ten Noever Bravw, M. C., Bovwman J., Gramberg L. G. and Lavos G. F. (1988), Compilation of Mass spectra of volatile compounds in Food., By TNO-CIVO Food Analysis Institute P. O. Box. 360, 3700 Aj Zeist, The Netherlands, Vol. **1–16**.
- Ueno H., Nakmuro K., Sayato Y. and Okada S. (1991), Characteristics of mutagenesis by glyoxal in *Salmonella typhimurium*: contribution of singlet oxygen. *Mutat. Res.* **251**, 99–107.