

## Evaluation of Antioxidant, Tyrosinase Inhibitory and Anti-inflammatory Activities of Turmeric(*Curcuma longa*) Extracts

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In this present paper, a preliminary evaluation on physiological activities of Turmeric(*Curcuma longa*) extracts is investigated. Initially, the DPPH radical scavenging activities of hot water and 95% ethanol extract of *C. longa* were measured  $58.24 \pm 0.058\%$  and  $39.20 \pm 0.021\%$  respectively, and tyrosinase inhibitory activities of hot water and 95% ethanol extracts were also measured  $43.14 \pm 0.028\%$  and  $93.14 \pm 0.021\%$ , respectively. In case of *C. longa* extracted by hot water, RAW 264.7 cell growth gradually increased with increasing concentration. But in the extracts of 95% ethanol, a RAW 264.7 cell has very good growth in a  $40 \mu\text{g/ml}$ , and its cell growth has tended to decrease in higher than  $40 \mu\text{g/ml}$  concentration. The inhibition rate of NO production in hot water and 95% ethanol extract was about 14.8% at  $80 \mu\text{g/ml}$ , and about 79.4% at  $100 \mu\text{g/ml}$  concentration. The microbial growths of *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhimurium* treated with hot water extract showed no inhibitory response, but growth of *S. pyrogenes* bacteria has about 18.2% inhibited in  $1,500 \mu\text{g/ml}$ . In case of 95% ethanol extract, microbial growth of 5 strains decrease depends on the concentration of the extract.

**Key words:** *Curcuma longa*, DPPH radical scavenging, tyrosinase inhibitory, anti-inflammatory.

### I. Introduction

*Curcuma longa* belong to the family of Zingiberaceae and is a perennial, tropical herb cultivated widely in Asia. It is primarily consumed in the form of powdered rhizomes mainly coloring, flavor to foods, with its associated medicinal properties, and it imparts preserves the freshness of the product prepared(Govindarajan and Stahl, 1980). Turmeric, the powdered rhizome, is reportedly used as an antiseptic, a cure for poisoning, to eliminate body waste products, for treating dyspepsia, and respiratory disorders, for some skin diseases, including wound healing(Ammon and Wahl, 1991;

Srimal, 1997), and as a cure for menstrual disorders, rheumatism and traumatic diseases due to the number of monoterpenoids, sesquiterpenoids, and curcuminoids it contains(Tang and Eisenbrand, 1992).

Extracts, as well as purified compounds from the rhizomes and aerial parts of *C. longa* have shown a number of biological activities including antimicrobial, anticancer, and topoisomerase-inhibitory activities, which have been reported as antioxidant and anti-inflammatory agents(Ammon and Wahl, 1991; Rao et al., 1995; Srimal, 1997; Roth et al., 1998). In specific, the volatile oil was isolated from turmeric rhizome which was analyzed

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by Gas chromatography–mass spectrometry and reported to contain 16 constituents of which, 6 compounds contributing 70.0% of the total oil constituents (Naz et al., 2010). In recent years, much attention has been focused on selective, plant-derived growth modulators in the human metabolic system (specially intestine), based on the fact that most plant-extracted materials are relatively nontoxic to humans (Kim et al., 2005a; Kim et al., 2005b). Therefore, in this study, we have evaluated the cytotoxicity on the RAW 264.7 cell line, antioxidant, tyrosinase inhibitory, anti-inflammation and antimicrobial activities of *C. longa* extracts.

## II. Material and Methods

### 1. Plant material and extraction

*Curcuma longa* was purchased from herb medicine store. Each sample was separately prepared by freeze drying, grinding, and finally stored at  $-70^{\circ}\text{C}$  conical tubes. The resulting powder of each plant (25 g) was extracted in 250 ml of hot water using rotary cooling evaporator for 5hr at  $100^{\circ}\text{C}$ , and in 125 ml of 95% ethanol using a soxhlet extractor for 3hr at  $80^{\circ}\text{C}$ , and then filtered. The solution obtained from each powder was concentrated under vacuum freeze drying.

### 2. DPPH free radical scavenging activity

An assay for DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging potential was performed as a method. The sample solution (200  $\mu\text{l}$ ) mixed with 100 mM Tris-HCL (pH 7.4) 800  $\mu\text{l}$  and afterwards added to a 500  $\mu\text{M}$  DPPH solution with 1 ml dissolved in methanol. After mixing incubation at room temperature for 20min, absorbance was then measured at 517 nm by UV-VIS spectrophotometer. BHA (butylated hydroxy anisole) and BHT (butylated hydroxy toluene) were used as positive controls and calculated as: Scavenging

activity(%) =  $[(A_0 - A_1) / A_0] \times 100$ , where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance in the presence of the test compound.

### 3. Tyrosinase inhibitory activity assay

Mushroom tyrosinase and L-tyrosinase were purchased from Sigma (St. Louis, MO, U.S.A) and assay were performed as a method. The reaction mixtures, consisting of 200  $\mu\text{l}$  of 0.175 M phosphate buffer (pH 6.8), 200  $\mu\text{l}$  of 5 mM L-DOPA, 100  $\mu\text{l}$  of mushroom tyrosinase (2,000 unit/ml) and 500  $\mu\text{l}$  of sample, were mixed in ependrop tube, and pre-incubated at  $35^{\circ}\text{C}$  for 2min. Optical densities were measured at 475 nm. Tyrosinase inhibition percent was calculated as follows: percentage(%) inhibition =  $(1 - (S - B / C)) \times 100$ , where, S=O.D. tyrosinase, sample and L-DOPA; B=O.D. without the tyrosinase; C=O.D. without the test sample; all O.Ds were determined at 475 nm.

### 4. Cell culture and test of cytotoxicity

The RAW 264.7 cell line was purchased from the Korean Type Culture Collection (KTCC). The cells were grown in a humidified incubator containing 5%  $\text{CO}_2$  and 95% air at  $37^{\circ}\text{C}$ . They were grown in EMEM (Dulbecco's modified Eagle's medium) from Sigma, supplemented with 10% FBS (fetal bovine serum) and a mixture of the following antibiotics: gentamicin, penicillin (100 unit/ml) and streptomycin (100  $\mu\text{g}/\text{ml}$ ) (all from Sigma), 1.5 g/L of sodium bicarbonate and in  $75\text{cm}^2$  tissue culture flasks. The culture medium was changed every 3 days, and the cells were usually split 1:3 when they reached confluence, and then treated with trypsin. Plates were changed to FBS-free medium before the beginning of the assay. For the measurement of cytotoxicity, MTT (3-(4,5-dimethylthiazoly-2)-2,5-diphenyltetrazolium bromide) assay was performed as previously described by Mossmann (1983). In brief, cells were seeded at  $1 \times 10^4$  cells/well in a 96-well plate and were treated with various concentrations (0–100  $\mu\text{g}/\text{ml}$ ) for