

Full Length Research Paper

## Antioxidant and anticancer activities of methanolic extract of *Trollius chinensis* Bunge

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The flower of *Trollius chinensis* Bunge (*Flos trollii*) is used for treating upper respiratory infections, pharyngitis, tonsillitis and bronchitis as a Chinese folk medicine since ancient times. This study aimed to investigate possible antioxidant and anticancer activity of methanolic extract of *F. trollii* (FTME). The antioxidant activity of FTME was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, hydroxyl radical scavenging assay and metal chelating assay. FTME exhibited a powerful free radical scavenging activity against DPPH and acted as a strong hydroxyl radical scavenger to prevent deoxyribose degradation in Fe<sup>3+</sup>/ascorbate/ethylenediaminetetraacetic acid (EDTA)/H<sub>2</sub>O<sub>2</sub> system. In addition, FTME also showed a weak metal chelating activity. Anticancer activity of FTME was determined by 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. FTME showed strong anticancer activities in antiproliferative activities against human gastric (AGS), A375SM, MCF-7, and MDA-MB-231 cancer cell lines. The half-inhibitory concentrations (IC<sub>50</sub>) for these cancer cells were 143.72, 62.23, 244.50 and 279.06 µg/ml, respectively. All results suggest that *F. trollii* is a powerful natural antioxidant, and also exert stronger anticancer activities for four kinds of human cancer cell lines. It could be a potential source of natural antioxidant and also as a health food.

**Key words:** *Trollius chinensis* Bunge, antioxidant, anticancer activity.

### INTRODUCTION

Free radicals were associated with serious disease such as diabetes, cirrhosis, cancer and cardiovascular diseases (Hertog et al., 1993; Zia-Ul-Haq et al., 2012a). Hence, a considerable number of investigations have been focused on the prevention of oxidative damage initiated by free radicals. In fact, much attention has been focused on the antioxidative compounds present in edible plants, because of some synthetic antioxidants, such as 2,3-*tert*-butyl-4-methoxy phenol (BHA) and 2,6-di-*tert*-butyl-4-methyl phenol (BHT) which are widely used in food industry (Imadia et al., 1983; Zia-Ul-Haq et al., 2008, 2011a). Those synthetic antioxidants were

deemed to have carcinogenic potential (Branen, 1975; Zia-Ul-Haq et al., 2011b). Some investigations showed that antioxidants from plant tissues are correlated with oxidative stress defense and serious different human disease, including cancer, arteriosclerosis and aging processes (Manosroi et al., 1995; Stajner et al., 1995; Zia-Ul-Haq et al., 2012b). The epidemiological investigations showed that more than 80% of cancers are connected to lifestyle. In addition, diet rich in fruits and vegetables are associated with a lower risk of several degenerative diseases (Franceschi et al., 1998). Therefore, investigations of natural plant antioxidants and

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related bioactive compounds for food preservation and certain human diseases have received much attention. *Trollius chinensis* Bunge, a member of Ranunculaceae's family, is a perennial herb widely distributed in north of China and Mongolia (Jiangsu New College of Medicine, 1977; Bai, 1994). Its flowers (*Flos trollii*), also called Jin Lianhua in China, as a traditional folk medicine since ancient times, have been used to treat colds, high fevers, aphthae, respiratory infections, chronic tonsillitis, pharyngitis, bronchitis and acute tympanitis in China (Jiangsu New College of Medicine, 1977; Kang et al., 1984). Presently, some studies have reported that the aqueous extracts of *F. trollii* exhibited the antimicrobial activities against the *Aureus*, *Pseudo-monas aeruginosa*, *Shigella dysenteriae*, *Escherichia coli*, *Streptococcus hemolyticus* and *Diplococcus pneumoniae*, and also showed antiviral activities against the coxsackie B3 (Wen et al., 1999), parainfluenza type 3 (Para 3) (Li et al., 2002) and influenza virus A (Cai et al., 2006). In addition, *T. chinensis* Bunge also showed a protective effect in D-galactose-aged mice (Fang et al., 2012). In this study, we investigated the possible antioxidative effects of *F. trollii* (FTME) and its anticancer activities against four kinds of human cancer cell lines *in vitro*.

## MATERIALS AND METHODS

### Reagents

Flower of *T. chinensis* Bunge (*F. trollii*) were purchased from a local market in Chongqing, China in August, 2011. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,3-*tert*-butyl-4-methoxy phenol (BHA), L-ascorbic acid, deoxyribose, trichloroacetic acid (TCA), ferrozine reagent, dimethyl sulfoxide (DMSO) and 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Roswell Park Memorial Institute (RPMI) 1640, Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). All other reagents were of analytical grade.

### Preparation of extracts

The flowers of *T. chinensis* Bunge (*F. trollii*) were shade dried initially, freeze dried and then ground to a fine powder. Ten grams of the powdered flower sample was then extracted for 24 h with methanol (200 ml) under continuous stirring at room temperature (28°C). Finally, all the methanolic extracts were evaporated to dryness at 50°C and redissolved in DMSO at a concentration of 50 mg/ml, and stored at -20°C prior to further use.

### DPPH radical scavenging assay

The DPPH radical scavenging activity of FTME was estimated according to the method of Hatano et al. (1988). The FTME (50, 100, 250, 500 µg/ml) were added to a methanolic solution (0.1 ml) of DPPH radical (final concentration of DPPH was 0.15 mM). The mixture was shaken vigorously and allowed to stand at room

temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm using a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer (Kyoto, Japan).

### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was carried out as described by Halliwell et al. (1987). The reaction mixture (1.4 ml) which contained FTME (0.2 ml, 50 to 500 µg/ml), deoxyribose (6 mM), H<sub>2</sub>O<sub>2</sub> (3 mM), KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> buffer (20 mM, pH 7.4), FeCl<sub>3</sub> (400 µM), ethylenediaminetetraacetic acid (EDTA, 400 µM), and ascorbic acid (400 µM), was incubated at 37°C for 1 h. The extent of deoxyribose degradation was tested by using the thiobarbituric acid (TBA) method. One milliliter of 1% TBA and 1 ml of 2.8% trichloroacetic acid (TCA) were added to the mixture, which was then heated in a water bath at 90°C for 20 min. The absorbance of the mixture was read spectrophotometrically at 532 nm using a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer (Kyoto, Japan).

### Metal chelating activity assay

The metal chelating activity of FTME on Fe<sup>2+</sup> was measured according to the method of Carter (1971). Briefly, FTME (50 to 500 µg/ml) were incubated with 0.05 ml of FeCl<sub>2</sub>·4H<sub>2</sub>O (2.0 mM). The reaction was initiated by the addition of 0.2 ml of ferrozine (5.0 mM) and finally quantified to 0.8 ml with methanol. After the mixture had reached equilibrium (10 min), the absorbance was read at 562 nm using a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer (Kyoto, Japan).

### Cell culture

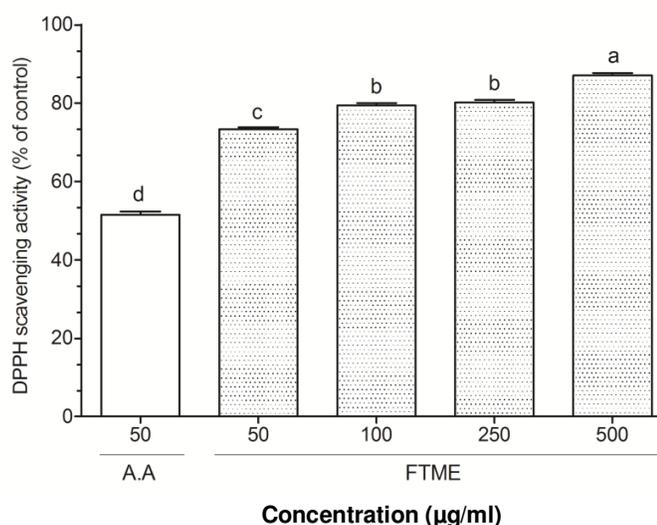
AGS, MCF-7, MDA-MB-231 and A375SM cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). The human gastric carcinoma cell lines AGS were grown in RPMI 1640 medium, and the human breast adenocarcinoma cell lines MCF-7, MDA-MB-231, and human melanoma cell lines A375SM were grown in Dulbecco's modified eagle's medium with L-glutamine; medium was supplemented with 10% FBS, 1% antibiotics in a humid atmosphere incubator (Model 3546, Forma Scientific Inc, Marietta, OH, USA) with 5% CO<sub>2</sub> at 37°C.

### Cell proliferation inhibitory assay

Cell proliferation inhibitory activity was measured using the MTT assay. Briefly, cancer cells were cultured in 96 well plates (Nunc, Rochester, NY, USA) at a density of 1 × 10<sup>4</sup> cells/ml and incubated with the FTME (50 ~ 500 µg/ml) for 48 h. After 48 h incubation, 100 µl MTT reagent (final concentration, 0.5 mg/mL) was added to each well, and cells were incubated in a humidified incubator at 37°C to allow the MTT to be metabolized. After 4 h, the media was removed and cells were resuspended in formazan in 100 µl of DMSO. The absorbance of the samples was measured at a wavelength of 490 nm by microplate reader (EL311, BIO-TEK Instruments, Inc, USA).

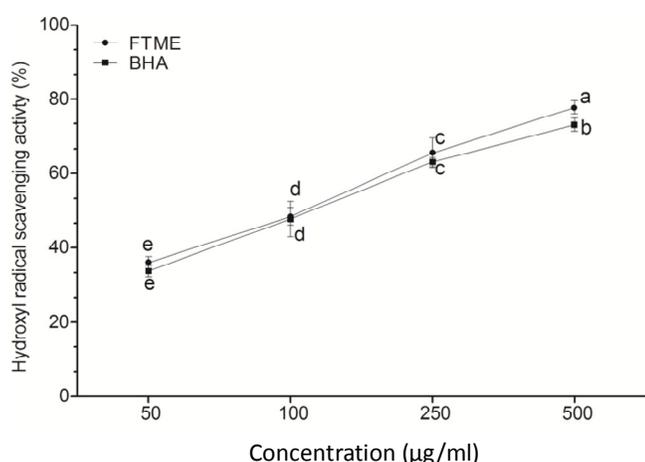
### Statistical analyses

Data were presented as mean ± standard deviation (SD). Differences between the mean values for individual groups were assessed by a one-way analysis of variance (ANOVA) with Duncan's multiple range tests. Differences were considered



**Figure 1.** DPPH free radical scavenging activity of *Flos trollii* methanolic extract (FTME).

Each value is expressed as mean  $\pm$  SD in triplicate. <sup>a-d</sup>Means with the different letters in the same column are significantly different ( $p < 0.05$ ) by Duncan's multiple range test. AA: ascorbic acid.



**Figure 2.** Hydroxyl radical scavenging activity of *Flos trollii* methanolic extract (FTME). Each value is expressed as mean  $\pm$  SD in triplicate. <sup>a-e</sup> Means with the different letters in the same column are significantly different ( $p < 0.05$ ) by Duncan's multiple range test.

significant when  $p < 0.05$ . The SAS v9.1 statistical software package (SAS Institute Inc., Cary, NC, USA) was used for these analyses.

## RESULTS AND DISCUSSION

### DPPH free radical scavenging activity

Free radicals play a very important role in several

physiological and pathological events, such as aging, inflammation, immunization, mutagenicity and carcinogenicity (Namiki, 1990). DPPH is a stable free radical (purple in colour) and accepts an electron or hydrogen radical to become a stable yellow diamagnetic molecule (Soares et al., 1997). As shown in Figure 1, the free radical scavenging activities of FTME were 73.29, 79.42, 80.19 and 87.05% at concentration of 50, 100, 250 and 500  $\mu\text{g/ml}$ , respectively. DPPH radical scavenging activities of FTME were increased in a manner dependent on concentration, which is comparable to the standard antioxidant ascorbic acid (51.49%) at 50  $\mu\text{g/ml}$ . The results suggested that FTME was a powerful antioxidant than ascorbic acid.

### Hydroxyl radical scavenging activity

The highly reactive hydroxyl ( $\cdot\text{OH}$ ) radicals can cause oxidative damage to DNA, proteins and lipids (Spencer et al., 1994), which contributes to carcinogenesis, mutagenesis and cytotoxicity. The effect of FTME on hydroxyl radical scavenging activity was determined by deoxyribose damage induced by  $\text{Fe}^{3+}$ /ascorbate/EDTA/ $\text{H}_2\text{O}_2$  system, and measured by the TBA method. Damaged deoxyribose degrades into fragments that react with TBA upon heating at a low pH to form a pink color. As shown in Figure 2,  $\cdot\text{OH}$  radical scavenging activities of FTME were 30.64, 48.42, 65.64 and 77.69 at concentration of 50, 100, 250 and 500  $\mu\text{g/ml}$ , respectively. The  $\cdot\text{OH}$  radical scavenging activities of BHA (positive antioxidant) were 33.71, 47.75, 63.15 and 73.05 at concentration of 50, 100, 250 and 500  $\mu\text{g/ml}$ , respectively. The  $\cdot\text{OH}$  radical scavenging activities of FTME were increasing in a concentration-dependent manner than that of BHA. These results clearly shows FTME can act as effective scavengers against hydroxyl free radical *in vitro*.

### Metal chelating activity

Lipid peroxidation is a very important biological consequence of oxidation cellular and aging in living organisms. Some metal ions, especially, ferrous iron can induce lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals (Fridovich, 1995; Halliwell, 1991). Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion, and reduce the catalyzing transition metal in lipid peroxidation (Hsu et al., 2003; Duh et al., 1999). Ferrozine can quantitatively form complex with ferrous ion. In the presence of chelating agents, the complex formation is disrupted with the result that the red color in the complex is decreased.

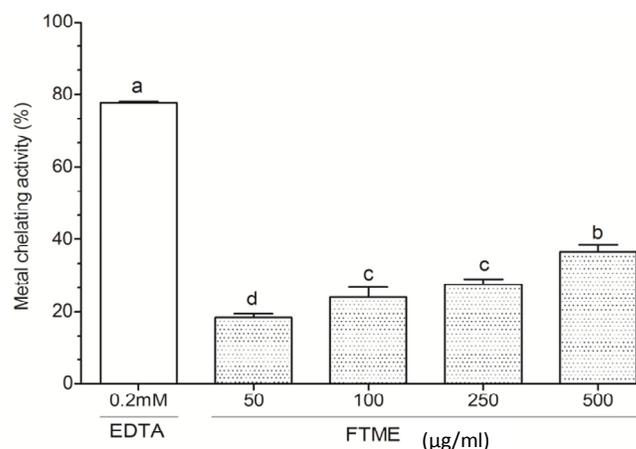
As shown in Figure 3, the metal chelating activities of FTME were 18.55, 24.13, 27.53 and 36.36% at concentration of 50, 100, 250 and 500  $\mu\text{g/ml}$ , respectively. However, the chelating effect activities of FTME were lower than that of EDTA (positive control) at 0.2 mM (chelating activity is 77.64%). FTME showed a weakly chelating ability of ferrous ions, which caused the lipid oxidation via Fenton reaction.

### Cell proliferation inhibitory effects

The cell proliferation inhibitory effects of FTME on different cancer cells, including AGS human gastric carcinoma cells, A375SM human melanoma cells, MCF-7 and MDA-MB-231 human breast adenocarcinoma cells were determined by MTT assay. Cancer cells were exposed for 48 h to various concentration of FTME (50 to 500  $\mu\text{g/ml}$ ). As shown in Figure 4, the cell proliferation inhibitory activities of FTME were increasing in a concentration-dependent manner. The half maximal inhibitory concentration ( $\text{IC}_{50}$ ) of FTME were 143.72, 62.23, 244.50 and 279.06  $\mu\text{g/ml}$  to against AGS, A375SM, MCF-7 and MDA-MB-231 cancer cells, respectively (Figure 4). In particular, FTME showed the strongest cell proliferation inhibitory activities than that of other cancer cells.

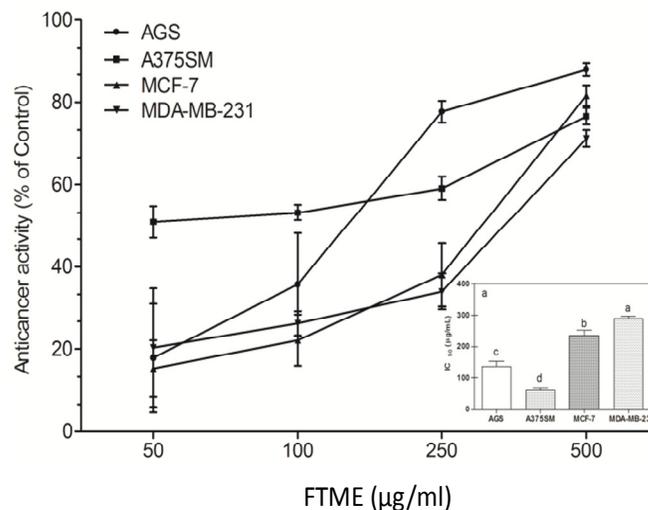
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**Figure 3.** Metal chelating activity of *Flos trollii* methanolic extract (FTME).

Each value is expressed as mean  $\pm$  SD in triplicate. <sup>a-d</sup>Means with the different letters in the same column are significantly different ( $p < 0.05$ ) by Duncan's multiple range test.



**Figure 4.** Cell proliferation inhibitory effects of *Flos trollii* methanolic extract (FTME) on AGS, A375SM, MCF-7 and MDA-MB-231 cancer cells.

Each value is expressed as mean  $\pm$  SD in triplicate. <sup>a-d</sup>Means with the different letters in the same column are significantly different ( $p < 0.05$ ) by Duncan's multiple range test.

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