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Full Length Research Paper

Study on antioxidant activity of *Echinacea purpurea* L. extracts and its impact on cell viability

Tzu Tai Lee[1], Chung Li Chen[2], Zhao Han Shieh[3], Jun Chen Lin[4] and Bi Yu[3]*

[1]Department of Biotechnology, Ming Dao University, Changhau, Taiwan 52345, Taiwan.
[2]Department of Agronomy, National Chung Hsing University, Taichung, Taiwan 40227, Taiwan.
[3]Department of Animal Science, National Chung Hsing University, Taichung, Taiwan 40227, Taiwan.
[4]Animal Industry Division, Council of Agriculture, Executive Yuan, Taipei, Taiwan 10014, Taiwan.

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This study investigates the antioxidant activity of *Echinacea Purpurea* L. (EP) extracts and its impact on cell viability. The polysaccharides content of EP was 159.8 ± 12.4 mg/g dry weight (DW), with extracts obtained by applying 55% ethanol at 55°C containing 11.0 ±1.0 mg gallic acid equivalent/g DW of total phenolic compound. Trolox equivalent antioxidant capacity, 0.1 mg/mL of EP extracts exhibited only 30% when compared to the ascorbic acid at the same concentration. Reducing power of extracts increased linearly with its concentration and the concentration at 2.0 mg/mL reached about 65% of ascorbic acid at 0.3 mg/mL. The chelating capacity of ferrous iron (Fe^{2+}) was 70% as good as that of the synthetic metal chelater EDTA when added to 5.0 mg/mL of EP extracts. The DPPH scavenging capacity showed 85.1% at 0.5 mg/mL of extracts and with half-effective doses (ED_{50}) was measured at 0.23 mg/mL. The superoxide anions scavenging capacity of EP extracts was nearly equivalent to ascorbic acid (91.1% vs 93.0%) at the same concentration of 1.6 mg/mL and ED_{50} was 0.32 and 0.13 mg/mL, respectively. Microculture tetrazolium assays showed extracts had 92% cell viability at 1.6 mg/mL for chicken's peripheral blood mononuclear cells (PBMCs) and 84% for RAW 264.7 macrophages, neither reaching the IC_{50} level. In summary, the EP extracts had antioxidant activity similar to that of ascorbic acid, but have no serious effect on inhibiting chicken's PBMCs viability.

Key words: Medicinal plant, *Echinacea purpurea* L., antioxidant, cell viability.

INTRODUCTION

Chinese medicinal herb, also known as herbal medium or botanical medium or phytomedium, generally refers to botanicals with medical effects and healthcare benefits. With proliferated knowledge and demand on natural health care, the World Health Organization (WHO), the US Food and Drug Administration (FDA) and European Union administration have independently announced their management regulations and relevant measures on traditional medicine and Chinese medicinal herb. The global demand for healthcare products based on Chinese medicinal herb is estimated to grow at 4.4% annually. The future market size is expected to be US$1.3 billion (The Freedonia Group, 2006).

The active ingredients of a medicinal plant are mainly its secondary metabolites, among which is the phenolic compound that is also an important antioxidant (Khanavi et al., 2009; Huda-Faujan et al., 2009). The phenolic compounds are generic term for multiple aromatic groups including mainly flavonoids, phenols acid, isoflavonoids and anthocyanins. These ingredients are naturally produced during a plant's growth metabolic process, the active substances with antioxidant function such as scavenging reactive oxygen species (ROS), free radicals (hydroxyl radicals, ·OH and superoxide anion radicals, $\cdot O_2^-$) or non-free radical reactive oxygen species (peroxide, H_2O_2) production from body metabolism

*Corresponding author. E-mail: byu@dragon.nchu.edu.tw. Tel: +886-4-22860799. Fax: +886-4-22860265.

Abbreviations: EP, *Echinacea purpurea* L.; **TEAC,** trolox equivalent antioxidant capacity; **ED_{50},** half-effective doses; **IC_{50},** half-inhibiting cell viability; **PBMC,** peripheral blood monouclear cells; **MTA_S,** microculture tetrazolium assays; **WSP,** water-soluble polysaccharide.

(Ramarathnam et al., 1995).

Echinacea purpurea L. (EP) is a kind of Asteraceae native perennial grown in North America normally used pharmacologically and for aesthetic enjoyment. Its root and subterranean stem were used by North America in early period to treat trauma and alleviate symptoms of infection and inflammation. The EP have been proven to show good immunoregulation and antiinflammation effects (Zhai et al., 2007) and with no hypersensitivity or other side effects during clinical trial stage (Saunders et al., 2007). As a result, the market has been seeing an escalating demand for EP from US$31 million in 1995 to US$80 million in 2005 globally. It is estimated to reach US$99 million in 2015 (The Freedonia Group, 2006).

Varieties of EP all contain similar main ingredients including caffeic acid derivatives, alkamides, flavonoids, essential oils, polyacetylenes of which the medical actives are yet to be exactly identified with corresponding diseases as of current applications (Thygesen et al., 2007). However, caffeic acid derivatives and alkamides have been proven to be ingredients with immunoregulation effects (Matthias et al., 2008). Moreover, synergistic antioxidative effect of caffeic acid derivatives, alkamides and polysaccharide fractions was demonstrated by measuring their inhibition of *in vitro* Cu(II)-catalyzed oxidation of human low-density lipoprotein (LDL) (Dalby-Brown et al., 2005).

For years, antibiotics have been popularly used in the animal industry. However, the misuse or continuous use of antibiotics has led to the emergence of the antibiotic-residue and drug-resistance (Monroe and Polk, 2000). With mounting public concerns associated with antibiotic-residues and increasing rates of antibiotic resistance, antibiotics have been strictly banned in some areas of the world. Addition of medicine herb to feed is one of the alternatives to be used as a replacement for antibiotics. There is sufficient evidence to show that potential herbs are effective for enhancement of the immune system and increasing antioxidant activity for human. Current study selects a medicinal plant with good potential to conduct assay on its antioxidant activity and cytotoxicity, with antioxidant activity assay further broken down to items of preventing oxidation (TEAC, reducing power and ferrous iron chelating capacity) and scavenging free radicals (such as DPPH and superoxide anion radicals), with a view to finding out antioxidant effect of EP active substances on the appraised items and understanding the influence of its extracts on cell viability (especially chicken peripheral blood mononuclear cells), in order to provide references for antibiotics substitution in broiler diet.

MATERIALS AND METHODS

Polysaccharides contents

Water-soluble polysaccharide (WPS) assay was conducted with the phenol-sulfate method. Boil dry EP powder in 95℃ for 2 h, with filtrate dialyzed under 4℃ for 12 h. The obtained extracts were analyzed with results compared to the data shown on the glucose standard curve at 730 nm spectrophotometrically (Dubois et al., 1956).

Antioxidant capacities

Plant material and extraction procedures

The plant of EP was obtained from Taichung District Agricultural Research and Extension Station, Council of Agriculture, Executive Yuan. The whole plants were dried out by cool air for 3 days and ground to fine powder (ca. 1mm size). Extract with 55% ethanol to distilled water (1:10, w/v) under 55℃ for 3 h after filtering (Advantec NO.1, Japan). The filtrate evaporated to dryness under vacuum. The further lyophilized extracts were added aqueous and adjusted to 1 mg/ml (original extracts) or 6 mg/ml (concentrated extracts) for following analyses.

Total phenolic contents

The total phenolic contents were determined using Folin-Ciocalteu reagent according to the method reported by Kujala et al. (2000). Mix Folin-Ciocalteu reagent with EP extracts evenly before adding the Na_2CO_3 solution and measure with a spectrophotometer at 730 nm. Then determine the contents of phenolic compounds of extracts as microgram of the gallic acid equivalent (GAE) by using an equation that was obtained from standard gallic acid graph.

Trolox equivalent antioxidant capacity (TEAC)

Trolox equivalent antioxidant capacity was measured as described by Gyamfi et al. (1999). The antioxidant assay kit was purchased from Cayman Chemical Co. Mix 0.25 mL of peroxidase (4.4 U/mL), 0.25 mL of 2, 2-azino-bis [3-ethyl-benzthiazoline-6-sulfonic acid] (ABTS, 100 μM), 0.25 mL of H_2O_2 (50 μM) and 1.5 mL of distilled water into the TEAC reagent. Absorbance was monitored at 750 nm for 5 min. Changes in absorbance were calculated and plotted with respect to concentrations of the against ascorbic acid and EP extracts. The final TEAC value was expressed as mM of Trolox antioxidant equivalent per gram.

Reducing power

The reductive capability of EP extracts was quantified by the method of Oyaizu (1986). Briefly, the EP extracts was mixed with phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide (1%). The mixture was incubated at 50℃ for 20 min. Then, the reaction was terminated by adding a portion of trichloroacetic acid (10%). The upper layer of solution was mixed the $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm in a spectrophotometer against an ascorbic acid was used as a control. Increased absorbance of the reaction mixture indicated greater reducing power.

Ferrous iron chelating capacity

The ferrous ions chelating activity of EP extracts and standards was investigated according to the method of Dinis et al. (1994). Briefly, EP extracts was added to a solution of 2 mM $FeCl_2$ and methanol. Then, the reaction was initiated by the addition of 5 mM ferrozine and mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm in spectrophotometer, wherein the Fe^{+2}

Table 1. Analysis of the total phenolic contents and soluble polysaccharides of *Echinacea purpurea* L. extracts[1].

Plant	Total phenolics (mg of GAE/g)	Soluble polysaccharides (mg/g DW)
Echinacea purpurea L.	11.0 ± 1.0	159.8 ± 12.4

[1]The value is expressed as mean ± standard deviation (n = 5).

chelating ability of EP extracts was monitored by measuring the ferrous ion-ferrozine complex at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was given in the below formula:

Ferrous ions chelating (%) = [(A0 - A1)/A0] x 100

where A0 was the absorbance of the control and A1 was the absorbance in the presence of the sample of EP extracts and EDTA. The control contains $FeCl_2$ and ferrozine, complex formation molecules.

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging capacity

The free radical scavenging activity of EP extracts was measured by DPPH using the method of Blois (1958). Briefly, 0.1 mM solution of DPPH in ethanol was prepared and added EP extracts at different concentrations (0 to 1.0 mg/mL). After 30 min, absorbance was measured at 517 nm and against ascorbic acid was used as a control. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and was calculated using the following equation:

DPPH scavenging effects (%) = 100 – [(A0 - A1/A0) x 100]

where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of EP extracts.

Superoxide anion radicals scavenging capacity

Superoxide anion scavenging activity of EP extracts was based on the method described by Nishimiki et. al., (1972). The superoxide radicals were generated in Tris-HCl buffer (16 mM, pH 8.0) containing 600 µM of nitroblue tetrazolium (NTB), 1872 µM of dihydromicotineamidadenibe dinucleotide (NADH) and sample solution of EP extracts were mixed. The reaction was further added by 240 µM of Phenazine methosulphate (PMS) and rests at room temperature for 5 min. The absorbance was measured at 560 nm in a spectrophotometer with ascorbic acid used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Inhibition superoxide anion generation (%) = 100 – [(A0 - A1)/A0] x 100

where A0 was the absorbance of the control (ascorbic acid) and A1 was the absorbance of EP extracts.

Cell viability test

Macrophage RAW 264.7 cells culture

The murine peritoneal macrophage RAW 264.7 cell line were purchased from Food Industry Research and Development Institute in Taiwan (BCRC No. 60001) and routinely cultured in 75 cm^2 flasks DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% bovine calf serum (HyClone, Logan UT), 100 µg/mL penicillin, 100 µg/mL streptomycin and 50 µg/mL amphotericin at 37°C in a 5% CO_2 mixed with 95% air incubator.

Peripheral blood mononuclear cells (PBMCs) isolation and culture

PBMCs were isolated from broilers and the blood was added by 1% EDTA for anticoagulant treatment, before being processed by the density gradient centrifugation. The PBMCs were separated out in histopaque®-1077 (Sigma, 10771) and then cultured in RPMI-1640 added 100 µg/mL penicillin, 100 µg/mL streptomycin and 50 µg/mL amphotericin at 37°C in a 5% CO_2 mixed with 95% air incubator (Kaiser et al., 2006).

Microculture tetrazolium assays (MTAs)

The trypan blue exclusion assay for cell growth and survival rate was based on Victoria et al. (1999). Suspensions of macrophage RAW 264.7 macrophages and PBMCs cell line at a density of 1 × 10^6 cell/well and were cultured at various concentrations in 10 uL of suspension in 96-wells microplate. After 48 h, 20 uL of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) solution was added to each well and the cells were incubated at 37°C for 5 h. Then, the medium was removed by aspiration and formazan crystals were dissolved in DMSO. Each well was completely pipetted and the absorption at 570 nm of formazan solution was measured using a micoplate reader. The absorbance of cell-free complete medium without EP extracts as a subtracted from the value of the corresponding treatment groups.

Statistical analysis

Results were the mean values of five times of the same sample and data represented as mean ± standard deviation (Mean ± SD).

RESULTS AND DISCUSSION

Polysaccharide contents

Polysaccharide contents of the EP was 159.8 ± 12.4 mg/g DW determined by phenol-sulfuric method (Table 1). Major polysaccharides present in the plant come from the cell wall and its metabolites. In addition to providing needs for the biological metabolism and energy, polysaccharides also exhibit anti-viral, anti-bacterial and anti-parasitic functions (Yang et al., 1998). Some polysaccharides that exhibit bio-activities are composed of

Table 2. Analysis of the TEAC value of *Echinacea purpurea* L. extracts[1].

Item	TEAC (mM trolox eq.)[1]
Ascorbic acid (0.1 mg/mL)	0.44 ± 0.11
Echinacea purpurea L. (0.1 mg/mL)	0.13 ± 0.05
Echinacea purpurea L. (0.8 mg/mL)	0.60 ± 0.07
Echinacea purpurea L. (1.6 mg/mL)	1.10 ± 0.03

[1]The value is expressed as ± standard deviation (n = 5).

monosaccharides with molecular weights ranging from 10^4 to 10^7. The complex and three-dimensional structures formed through the clustering of β-glycoside linkages. As indicated by Tsiapali et al. (2001), β-1-3-D-glucan and its derivatives not only have the immune-stimulating activity, but also possess various levels of free radical scavenging capacity. The mesmeric β-glucan unit was ten-fold stronger than the monomeric counterpart at the same concentration level (Patchen et al., 1987). *Astragalus membranaceus*, known as Radix, is a type of Chinese herb medicine, with the polysaccharide content at 101 mg/g. Although no significant positive effect on the growth performance was observed, it enabled the improvement in the immune regulation and stimulation in broiler (Guo et al., 2004). Polysaccharide contents are influenced by different cultivation conditions. The polysaccharide content of *Lentinus edodes* is 72 ± 4 mg/g using the general commercial cultivation media. However, the polysaccharide content can be increased to 410 ± 72 mg/g if switching to the whey and permeate-based culture media. Further, the polysaccharide content at 10 days will be twice as much than being cultivated at 20 days, indicating that polysaccharide contents were dependent on the growth and metabolic processes in plant (Wu and Hansen, 2008).

Total phenolic compound contents

The total phenolic contents of EP extracts contained 11.0 ± 1.0 mg gallic acid equivalent (GAE)/g DW (Table 1). Hudac et al. (2007) reported that *Echinacea* flower heads and leaves have higher phenolic contents, they suggested the result is the function of development stage. Prakash et al. (2007) discovered that the total phenolic contents of the twenty-five Chinese herb medicines being analyzed ranged from 2.8 mg GAE/g DW (*Withania somnifera*, root) to 107.8 mg GAE/g DW (*Cassia fistula*, fruit). Among the Chinese herbs, the highest total phenol content was also found in the fruit (including the flowers), while the roots exhibited the lowest level. Phenolic compounds are widely found in the plants as the secondary metabolites and the contents can be used as a critical index for determining the antioxidant capacity (Castelluccio et al., 1995; Khanavi et al., 2009). The antioxidant mechanism adopted by these phenol compounds is the direct reaction with free radicals, such as hydroxyl (·OH) radicals, superoxide anion (O2·-) radicals and hydrogen peroxide (H_2O_2, as oxygen in non-free radical state) for minimizing the damage to the cells and inhibiting lipid oxidation. Moreover, the preventive effects are thus generated for cardiovascular diseases, cancer, aging and cranial nerve disorders (Parr and Bolwell, 2000). To lower cellular damages caused by the free radicals, cells possess non-enzymatic (ascorbic acid, α-tocopherol, glutathione) and enzymatic (glutathione peroxydase, catalase, superoxide dismutase) antioxidant defense systems. Under a normal functional state, a dynamic balance will be achieved through the interactions between the organism's antioxidant system and the formation of free radicals or active oxygen products. However, an imbalance in the oxygen-reducing defense system can be observed when aging continues and environmental fluctuations occur. Thus, to enhance the comprehensive defense mechanisms, the increase in the body's glutathione peroxydase content and antioxidant replenishments, for instance, can thus sustain the balance (Ramarathnam et al., 1995). The total amount of the phenolic compounds contained in the Chinese herb medicine is not only affected by the species variations but also by the cultivation conditions and harvest season (Wu and Hansen, 2008; Orhan et al., 2009). Furthermore, the polarity or non-polarity properties of the solvent used for determining the total amount of phenolic compounds will also affect the extraction quantity. For example, solvents with polar activities, such as the methanol or water, often result in more optimal extraction quantity than non-polar counterparts such as hexane (Kang et al., 2003).

Trolox equivalent antioxidant capacity (TEAC)

The trolox equivalent antioxidant capacity of the EP extracts increased proportionally with the increasing concentration (Table 2). The TEAC exerted by the 0.1 mg/mL EP extracts was equivalent to 30% of the TEAC exhibited by the ascorbic acid at the same concentration. At 0.8 mg/mL, its antioxidant capacity outperformed the ascorbic acid with the concentration of 0.1 mg/mL. The TEAC shown by the EP extracts at 1.6 mg/mL was approximately twice as strong as at 0.8 mg/mL (1.1 vs 0.6 mM trolox eq., Table 2). It therefore demonstrated that its antioxidant capacity exhibited a liner relation under such concentration. The TEAC method was dependent on the reactions between the antioxidants and oxidants. Due to the fact that the analysis was centered on the final reaction products, it was less influenced by the reaction rate as compared to the ferric ion reducing antioxidant (FRAP) method (Huang et al., 2005).

Reducing power

The reducing power shown by the EP extracts are illu-

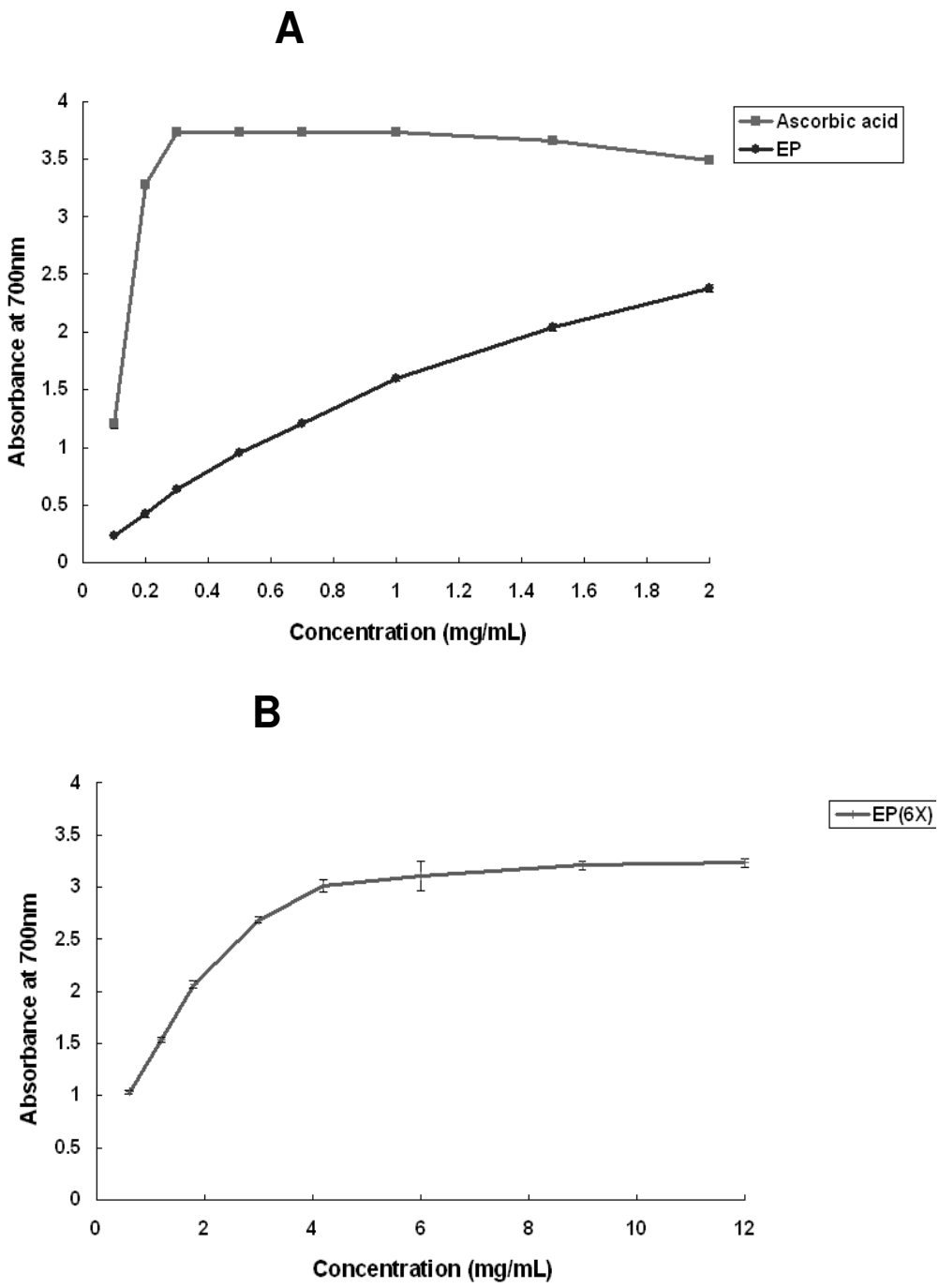

Figure 1. Reducing power of *Echinacea purpurea* L. extracts and ascorbic acid (A) and six-fold concentrate (B). Each value represent Mean ± SD (n = 5).

strated in Figure 1A. The ascorbic acid attained the maximum reducing power when the concentration increased from 0.1 to 0.3 mg/mL. Subsequently, the reducing power (A_{700} value) did not further increase proportionally to the increase in the concentration. The reducing power of the EP extracts showed a liner increase (y = 1.1292x + 0.2934, x = concentration of EP extracts) as the concentration went up to 2 mg/mL. In Comparing ascorbic acid and EP extracts, the reducing power was equivalent when the concentrations were 0.1 and 0.7 mg/mL, respectively. When the EP extracts concentration was 2.0 mg/mL, the reducing power was equivalent to 65% of the reducing power exhibited by the ascorbic acid at the same concentration. After being six-fold concentrated extracts (equally 6 mg/ml), the EP extracts presented an absorbance value of 3.01 at 4.2 mg/Ml (Figure 1B),

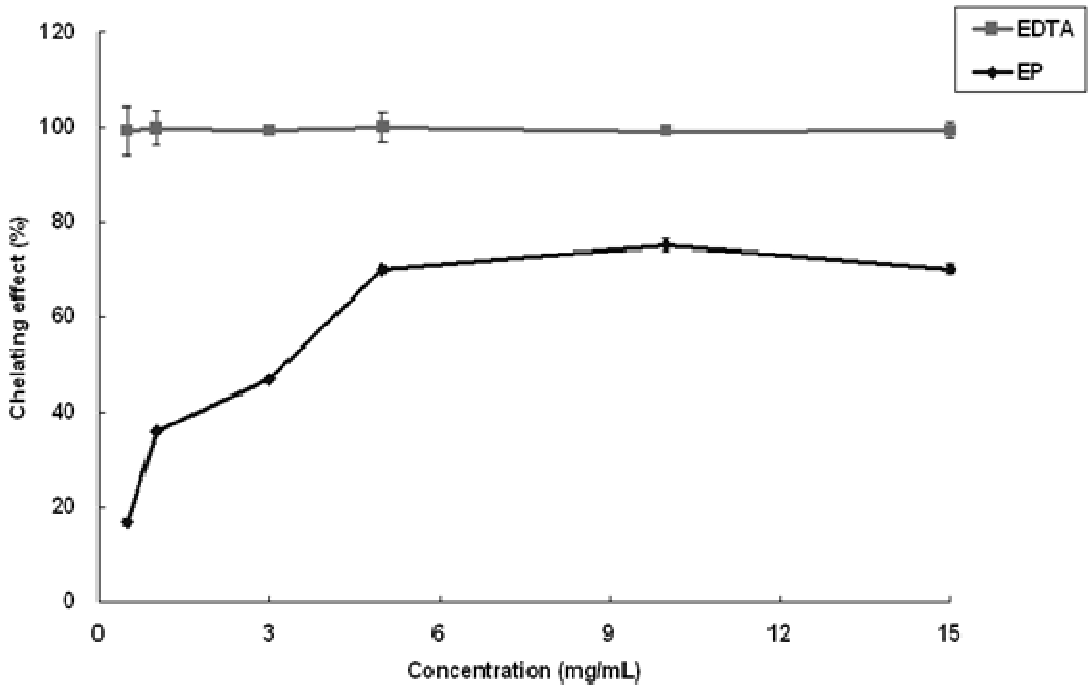

Figure 2. Chelating capacity of *Echinacea purpurea* L. extracts and EDTA on Fe^{2+}. Each value represent Mean ± SD (n = 5).

indicating a high reducing power at such concentration. Even after the concentration was further increased, the reducing power was still limited. At the concentration below 1.5 mg/mL, the EP extracts exhibited an absorbance value approximately one-fifth to one-sixth of that for the concentrated solution. As a result, it indicated a linear correlation between the concentration range and the reducing power represented by the absorbance value. When the six-fold concentrate was converted to be approximately equivalent to the 4.2 mg/mL of the original extracts, the reducing power will attain the maximum level.

Ferrous iron chelating capacity

As illustrated in Figure 2 for the ferrous iron chelating ability. The EP extracts exerted an equivalent of 70% chelating effect at 5.0 mg/mL when compared with the EDTA at a lower concentration (0.5 mg/mL). The chelating ability was only increased slightly by 5% when the concentration was increased to 10 mg/mL, indicating a saturation state was almost attained at the 5.0 mg/mL of EP extracts. Free radicals were produced by the metal ions through the redox cycle and promoted acceleration of the lipid oxidation reaction. Thus this method only gained insight into its inhibitory effects on the free radical formation (Gordon, 1990). Ferrous ions were most frequently considered as the oxidation catalyst among various metals, causing the lipid oxidation acceleration. Therefore, it is usually used as an antioxidant assessment index (Ebrahimzadeh et al., 2008; Yamaguchi et al., 1988).

1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide anion radicals scavenging capacity

The DPPH and superoxide anion radicals scavenging ability and presented by the EP extracts are illustrated in Figure 3. The DPPH scavenging ability increased proportionally with the increase in the concentration range from 0 to 0.5 mg/mL (Figure 3A). In contrast with the 87.6% DPPH scavenging ability generated by the ascorbic acid at 0.016 mg/mL, the BHT (butylated hydroxytoluene) concentration needed to be at 0.125 mg/mL for achieving the 91.3% scavenging ability. The EP extracts showed 85.1 and 91.4% scavenging abilities at 0.5 and 1.0 mg/mL, respectively. The half-effective dose (ED_{50}) was calculated at 0.23 mg/mL. Since the DPPH is a relatively stable artificial free radical, it is often used to evaluate the antioxidant's free radical scavenging ability by measuring the absorbance value to determine the reduced quantity, which is produced by the reduction of the DPPH by the hydrogen ions supplied by the antioxidant. However, if the extracts contain excessive anthocyanins, it might also indicate an interference with the DPPH analysis values and leads to underestimation of antioxidant activity (Arnao, 2000; Awika et al., 2003; Hudec et al., 2007).

The superoxide anion radicals scavenging capacity of the EP extracts is as shown in Figure 3B. When EP

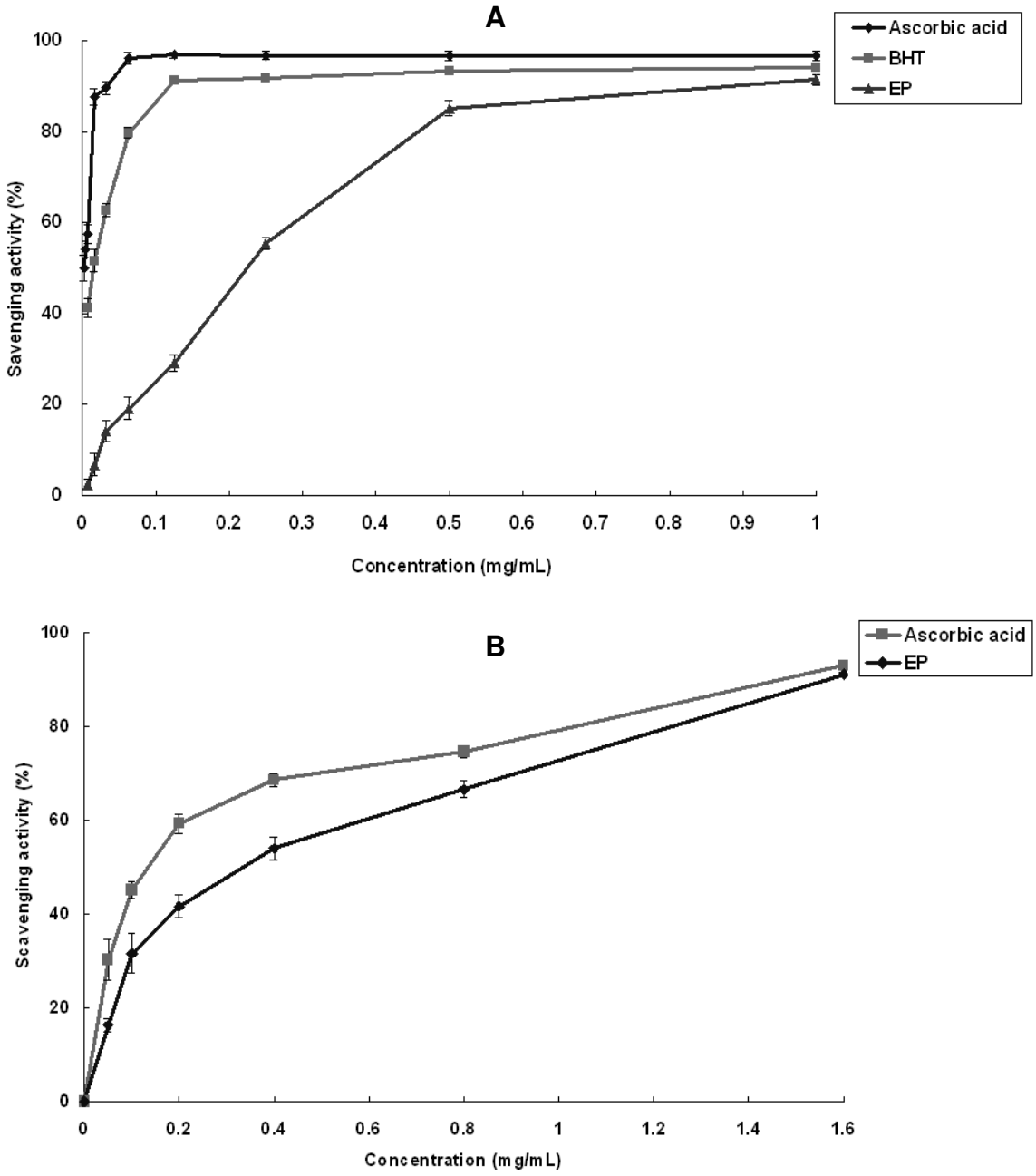

Figure 3. Scavenging activity of *Echinacea purpurea* L. extracts, ascorbic acid and BHT on DPPH (A) and superoxide anion radical (B). Each value represent Mean ± SD (n = 5).

extracts and ascorbic acid concentrations were 0.2 mg/mL, respective scavenging abilities were 41.7 and 59.3%. The scavenging ability was further enhanced proportionally with the EP extracts concentration. The superoxide anion scavenging abilities of the extracts and ascorbic acid stood at equal levels (91.1 vs 93.0%) when the concentration was at 1.6 mg/mL, with the respective ED_{50} values at 0.32 and 0.13 mg/mL. Gulcin (2005) analyzed the superoxide anion scavenging ability of the black pepper seed and used the ascorbic acid as a control candidate. The results showed that the scavenging abilities of pepper seeds, upon being extracted by water and ethanol, were respectively 64.2 and 22.6% of that for the ascorbic acid when the concentration of water-extracts and ethanol-extracts were at 0.075 mg/mL. In comparing EP extracts, the concentration reached below 0.05 mg/mL, fifty percent of the ascorbic acid's scavenging ability was attained, indicating that EP extracts had a better superoxide anion scavenging ability than the black pepper seeds extracts.

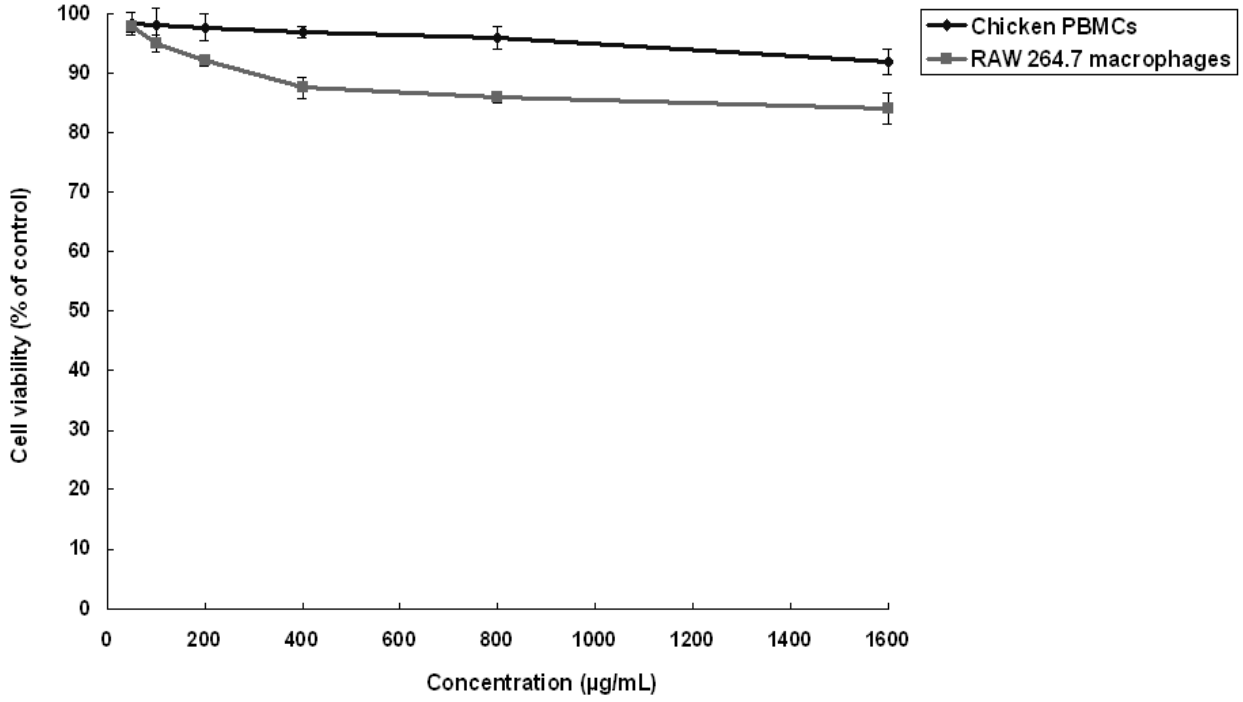

Figure 4. Effect of *Echinacea purpurea* L. extracts on cell viability of chicken's peripheral blood monouclear cells (PBMCs) and RAW 264.7 macrophages for 24 h incubation.

Cell viability assay

As illustrated by the cell viability assay in Figure 4, the survival rate (%) of the chicken's peripheral blood mononuclear cells (PBMCs) was much better than RAW 264.7 macrophages under different concentrations of EP extracts. When the concentration was up to 1.6 mg/mL, the survival rates (%) of the PBMCs and RAW 264.7 macrophages were 92 and 84%, respectively. Both values did not satisfy the cell viability inhibition standards defined by IC_{50}. Results showed that the negative effect of EP extracts on the PBMCs was relatively less significant than on the RAW 264.7 macrophages. The MTAs method is currently and mostly used for the assessments specifically on cyto-toxic competency. The evaluation of the potential inhibitory effect from the sweet potato extracts on the cell viability of the human leucocytes NB4 was conducted by Huang et al. (2004). However, it is less targeted at investigating and comparing survival rates of normal animal cells. For the current study, the PBMCs were the investigation candidates for enhancing application feasibility on the organisms.

Anti-oxidation assays assessment methods can generally be classified into the hydrogen atom transfer (HAT) and electron transfer (ET) methods based on the response mechanisms. The antioxidant activity evaluation done by this study was primarily based on the electron transfer methods. Electron transfer assessment categories mainly encompassed the total phenolic contents, TEAC, DPPH scavenging ability, superoxide free radical scavenging capacity and reducing power. Awika et al. (2003) compared the methods used by DPPH scavenging, TEAC and total phenolic contents with the antioxidant capacities of the *Sorghum bicolor* and its extracts. The results identified that the antioxidants contained in the *S. bicolor* exhibited a level of uniformity trend among the methods being evaluated ($R^2 > 0.96$), indicating that those methods could all elaborate the antioxidant capacities of the supply and tested materials.

Conclusion

In summary, the combined results of the study show that the medicinal plant EP extracts have good capability for oxidation prevention and display antioxidant effect for scavenging free radicals, but with no effect on inhibiting chicken PBMCs viability at concentration under the detected. It is assayed that the optimal concentration is 4.2-4.5 mg/mL for producing best reducing power and ferrous iron chelating capacity and the ED_{50} of scavenging capacity for DPPH and superoxide anion radicals is about 0.23 - 0.32 mg/mL.

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