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Free radical and reactive oxygen species scavenging activities of peanut skins extract

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Abstract

Peanut skin, a low economic value by-product of the peanut industry, is rich in potentially health promoting compounds. The major objective of this study was to investigate free radical and reactive oxygen species scavenging activities of water soluble extracts from defatted peanut skins (EPS) employing various in vitro assay systems. The total phenolic content of EPS was 0.097 g gallic acid equivalents/g of skin tested, and the total flavonoid content of EPS was 0.065 g catechin equivalents/g of skin tested. EPS at 500 μg/ml exhibited 97.1% of DPPH radical scavenging activity and 98.6% of inhibition of erythrocyte hemolysis, and showed 98.6%, 89.1% and 85.3% of scavenging activities on $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, respectively, and also effectively exerted 76.6% of Fe^{2+} chelating activity. These results suggested that EPS might be beneficial as a potent antiradical and antioxidant and effectively employed as an ingredient in food applications.

Keywords: *Arachis hypogaea*; Free radical scavenging; Peanut skins; Reactive oxygen species

1. Introduction

Free radicals and other reactive oxygen species including superoxide anion radicals ($O_2^{\cdot-}$), hydroxyl radicals (OH) and hydrogen peroxide ($H_2O_2^{\cdot}$) are highly reactive and potentially damaging transient chemical species formed in aerobic life. These unwanted metabolic by-products of normal aerobic metabolism are removed by a variety of endogenous reactive oxygen species scavenging enzymes and chemical compounds (Halliwell & Gutteridge, 1990). An imbalance between reactive oxygen species generating and their scavenging systems in cells leads to oxidative stress, resulting in some oxidative damage to biomolecules such as lipids, nucleic acids, proteins and carbohydrates (Sies, Stahl, & Sevanian, 2005). Oxidative damage increases the risk of several human chronic diseases, such as cancer, arteriosclerosis, neurodegenerative disorders, as well as aging processes (Aruoma, 1998). Over the last decade, growing scientific evidence suggests that polyphenols used as antioxidants may protect cell constituents against oxidative damage and, therefore limit the risk of various degenerative diseases associated with oxidative stress (Scalbert, Manach, Morand, & Rémésy, 2005).

Epidemiological studies suggest that frequent consumption of peanuts may reduce the risk of coronary heart disease (Fraser, Sabate, Beeson, & Strahan, 1992) and certain types of cancers (Awad, Chan, Downie, & Fink, 2000). The beneficial health effects might mainly be ascribed to the bioactive compounds in peanuts, such as flavonoids, plant sterols and resveratrol (Pennington, 2002). So far, numerous polyphenolics and related compounds, such as luteolin (Duh, Yeh, & Yen, 1992), proanthocyanidins (Lou et al.,

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1999), resveratrol (Sanders, McMichael, & Hendrix, 2000), flavonoids (Lou, Yuan, Yamazaki, Sasaki, & Oka, 2001), ethyl protocatechuate (Huang, Yen, Chang, Yen, & Duh, 2003), *p*-coumaric acid and its esterified derivatives (Talcott, Passeretti, Duncan, & Gorbet, 2005) have been identified in peanuts.

Peanut is the pod, or legume, of *Arachis hypogaea* L., of the family Leguminosae, which originated in South America and is cultivated around the world in tropical, sub-subtropical and warm temperate climates (Stalker, 1997). On a worldwide scale, peanut is grown primarily for its seed oil. Peanut is also an important food source of protein in developing and developed countries. Peanut comprises kernels, skins (seed testae, seed coats) and hulls. Peanut skins and hulls are extremely low economic value by-products of peanut processing operations that remain underutilized. In recent years, several investigations were conducted to study antioxidant properties of peanut, peanut kernels, peanut hulls and peanut-based products. Peanut kernel has been reported to contain antioxidant flavonoids, dihydroquercetin (Pratt & Miller, 1984). It has been reported that the methanolic extracts from peanut hulls have both strong antioxidant activity (Duh & Yen, 1995) and properties of scavenging free radical and reactive oxygen species (Yen & Duh, 1994). Hwang, Shue, and Chang (2001) reported that roasted and defatted peanut kernels showed remarkable antioxidative activity on linoleic acids in emulsions. Nepote, Mestrallet, and Grosso (2004) reported that the extracts from peanut skins in honey-roasted peanuts inhibited lipid oxidation.

Peanut skins consist of skins from processed peanuts, broken nuts and, sometimes, nuts that may have been rejected during the preparation of peanuts for human consumption. Peanut skins were demonstrated to be rich in phenolics and potentially other health promoting compounds and to be free of compounds that are toxic to animals, which can be extracted for use in food applications (Sobolev & Cole, 2003). Peanut skins have long been used in China as a traditional Chinese medicine for the treatment of chronic haemorrhage and bronchitis. Recently, very few works have been undertaken to study the antioxidative compounds obtained from peanut skins. These few studies showed the water soluble extract of peanut skins containing proanthocyanidins and flavonoids suppressed protein glycation (Lou et al., 2001) and possessed substantial activity against hyaluronidase (Lou et al., 1999). However, to the best of our knowledge, there is limited literature on the free radical and reactive oxygen species scavenging activities of the water soluble extract of peanut skins (EPS). The objectives of this work were to investigate free radical and reactive oxygen species scavenging activities of the water soluble EPS employing various in vitro assay systems, namely, 2,2-diphenyl-1-1picrylhydrazyl (DDPH) radical, superoxide anion ($O_2^{-\cdot}$), hydroxyl radical (OH) and hydrogen peroxide ($H_2O_2^{\cdot}$) scavenging activities, Fe^{2+} chelating activity, and inhibition of hemolysis of normal human erythrocyte induced by peroxyl radicals, in order to understand the mechanisms of its antioxidative activity.

2. Materials and methods

2.1. Material

Peanut skins purchased from a local herbal pharmacy were kept in a sealed plastic bag and stored at 4 °C until use. Ascorbate, 2,2′-azobis-2-amidinopropane dihydrochloride (AAPH), butylated hydroxyanisole (BHA), catechin, 2-deoxy-D-ribose, 2,2-diphenyl-1-1picrylhydrazyl (DPPH), nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), xanthine, xanthine oxidase were purchased from Sigma-Aldrich (St. Louis, MO). Hydrogen peroxide, disodium ethylenediamine tetracetate (EDTA), trichloroacetic acid and ferric chloride were purchased from Shanghai chemical agents company, China. All other chemicals and solvents used were of analytical grade.

2.2. Preparation of EPS

A 50 g sample of peanut skins were defatted with *n*-hexane (3 × 400 ml) for 10 h at room temperature, and the *n*-hexane remained in peanut skins was removed under reduced pressure in a vacuum oven. The defatted peanut skins (20.0 g) were macerated overnight with 300 ml aqueous ethanol (50% v/v) in a shaking incubator (150 rpm) at room temperature in darkness. The extract was filtered and the residue was extracted again under the same conditions. The combined filtrates were evaporated to a final volume of 10 ml under vacuum in a rotary evaporator (RE-52AA Model, Yarong Biochemical Instruments Plant of Shanghai, China) at 35 °C. The concentrated solution was lyophilized with a freeze dryer system (AIPHA 1–4, Christ, Germany) to obtain the peanut skin extract. The dried extract was weighted to determine the yield based on peanut skins dry basis. Data are reported as means ± standard deviation (SD) for three replications.

2.3. Determination of total phenolic content

The total phenolic content of EPS was determined with the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, EPS (3 mg) was dissolved in 10 ml of distilled water. An aliquot of 100 μl of appropriate dilution of sample extract was shaken for 1 min with 500 μl of the Folin–Ciocalteu reagent freshly prepared in our laboratory, and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% (w/v) sodium carbonate was added and the mixture was shaken once again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 2 h of reaction at ambient temperature, the absorbance at 750 nm was evaluated using glass cuvettes. Using gallic acid as standard, the total phenolic content of peanut skins was expressed as a gallic acid

equivalent (g gallic acid/g skin). Data are reported as means ± SD for at least three replications.

2.4. Determination of total flavonoid content

Total flavonoid content was determined by a colorimetric method described by Jia, Mengcheng, and Wu (1999) with minor modification. An aliquot of 10 ml of appropriate dilution of EPS was added to volumetric flask containing 1 ml of 5% (w/v) sodium nitrite and placed for 6 min, followed by reaction with 1 ml of 10% (w/v) aluminum nitrate to form a flavonoid–aluminum complex. After 6 min, 10 ml of 4.3% (w/v) NaOH was added and the total was made up to 25 ml with distilled water. After 15 min at room temperature, the final solution was mixed well again and the absorbance was measured against a blank at 510 nm with a UV-2100 UV/VIS Recording Spectrophotometer (UNICO (Shanghai) Instruments Co., Ltd., China). The total flavonoid content of peanut skins was expressed as a catechin equivalent (g catechin/g skin). Data are reported as means ± SD for at least three replications.

2.5. Scavenging activity of DPPH radical

The scavenging activity of EPS on DPPH radicals was measured according to the method of Yuan, Wang, Yao, and Chen (2005) with minor modification. BHA was used as a reference material. Briefly, an aliquot of 0.2 ml of sample solution at different concentrations (10–500 μg/ml) was mixed with 0.8 ml of Tris–HCl buffer (100 mM, pH 7.4) and 1.0 ml of 0.5 mM methanolic solution of DPPH. The control contained all the reaction reagents except EPS or BHA. The reaction mixture was shaken well and incubated for 30 min in the darkness at room temperature, and the absorbance of the resulting solution was measured at 517 nm with a UV-2100 UV/VIS Recording Spectrophotometer. The radical scavenging capacity of the tested samples was measured as a decrease in the absorbance of DPPH radical and was calculated by Eq. (1). All determinations were performed in triplicate

$$\text{Scavenging capacity } (\%) = (1 - A_{\text{samples}}/A_{\text{control}}) \times 100. \quad (1)$$

2.6. Metal ions chelating activity

The ferrous ion chelating potential of EPS was measured according to the method of Yamaguchi, Ariga, Yoshimura, and Nakazawa (2000) with minor modification. EDTA was used as reference material. Briefly, 0.25 ml of $FeSO_4$ solution (1 mM) and an equal volume of test sample solution at different concentrations (10–500 μg/ml) were mixed; 1 ml of Tris–HCl buffer (pH 7.4) and 2,2′-bipyridyl solution (0.1% (w/v) in 0.2 M HCl), were added to the mixture, respectively, together with 0.4 ml of 10% (w/v) hydroxylamine-HCl and 2.5 ml of ethanol. The control contained all the reaction reagents except the tested samples. The reaction mixture was adjusted to a final volume of 5 ml with water and shaken well and incubated for 10 min at room temperature. The absorbance was determined at 522 nm. A lower absorbance of the reaction mixture indicated a higher ferrous ion chelating ability. The capability to chelate the ferrous iron was calculated by the following:

$$\text{Chelating effect } (\%) = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100. \quad (2)$$

All determinations were performed in triplicate.

2.7. Hydroxyl radical-scavenging assay

The hydroxyl radical-scavenging ability of EPS was determined using 2-deoxy-D-ribose oxidative degradation mediated by hydroxyl radicals as described by Halliwell, Gutteridge, and Aruoma (1987) with slight modification. The reaction mixture, which contained EPS (0.5 ml, 10–500 μg/ml), 0.1 ml of 2.8 mM 2-deoxy-D-ribose, 0.2 ml of a premixed 100 μM $FeCl_3$, and 104 μM EDTA solution (1:1, v/v), 0.1 ml of 1 mM hydrogen peroxide and 0.1 ml of 0.1 mM ascorbic acid, was incubated at 37 °C for 60 min. Thereafter, 1.0 ml of 0.5% (w/v) thiobarbituric acid in 10% (w/v) trichloroacetic acid were added and the mixtures were vortexed and heated in a water bath at 100 °C for 15 min. The reaction was stopped by a 5 min ice water bath. The mixtures were centrifuged at 12,000*g* for 5 min at room temperature, and the absorbance of the supernatants were measured at 532 nm. BHA was used as the reference material. The scavenging effect of hydroxyl radicals (%) was calculated using the following:

$$\text{Scavenging effect } (\%) = (1 - A_{\text{samples}}/A_{\text{control}}) \times 100. \quad (3)$$

where A_{samples} was the absorbance in the presence of the tested samples, and A_{control} was the absorbance of the control contained all the reaction reagents except the tested samples.

2.8. Superoxide anion radical-scavenging assay

The superoxide anion radical scavenging activity of EPS was assessed spectrophotometrically using the xanthine/xanthine oxidase method described by Okamura, Mimura, Yakou, Niwano, and Takahara (1993) with slight modification. The reaction mixture, which contained EPS (0.5 ml, 10–500 μg/ml), 1.0 ml mixture of 0.4 mM xanthine and 0.24 mM NBT in 0.1 M phosphate buffer (pH 8.0) and 1.0 ml solution of xanthine oxidase (0.049 U/ml) diluted with 0.1 M phosphate buffer (pH 8.0), was incubated in a water bath at 37 °C for 40 min. The reaction was terminated by adding 2.0 ml of an aqueous solution of 69 mM sodium dodecylsulfate and the absorbance of the reaction mixture was measured at 560 nm. Decreased absorbance of the reaction mixture indicates increased superoxide anion radical scavenging activity. BHA was used as positive control. The scavenging effect of superoxide anion was calculated by the following:

$$\text{Scavenging effect } (\%) = (1 - A_{\text{samples}}/A_{\text{control}}) \times 100 \quad (4)$$

where A_{samples} was the absorbance in the presence of the tested samples, and A_{control} was the absorbance of the control without the tested samples.

2.9. Hydrogen peroxide-scavenging assay

The hydrogen peroxide scavenging ability of EPS was determined according to the method described by Ruch, Cheng, and Klauning (1989). A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (0.1 mM, pH 7.4) at 20 °C. Hydrogen peroxide concentration was determined spectrophotometrically at 230 nm using a molar extinction coefficient of hydrogen peroxide of 81 M^{-1} cm^{-1} (Beers & Sizer, 1952). An aliquot of 3.4 ml of sample dissolved in phosphate buffer (0.1 mM, pH 7.4) with various concentrations (10–500 μg/ml) was mixed with 600 μl of a hydrogen peroxide solution. BHA was used as the reference compounds. The concentration of hydrogen peroxide was measured by reading the absorbance values at 230 nm of the reaction mixtures at 10 min against a blank solution containing in phosphate buffer without hydrogen peroxide. The scavenging capacities of hydrogen peroxide of both sample and standard compounds were calculated using the following:

$$\text{Scavenging effect } (\%) = (1 - A_{\text{samples}}/A_{\text{control}}) \times 100 \quad (5)$$

where A_{samples} was the absorbance in the presence of the tested samples, and A_{control} was the absorbance of the control without the tested samples.

2.10. Assay for normal human erythrocyte hemolysis induced by peroxyl radicals

The in vitro inhibition of normal human erythrocyte hemolysis of EPS was assessed according to the procedures described by Yuan, Wang, and Yao (2005) with some modifications. Human blood from healthy donors was obtained from the local Red Cross Blood Branch. Blood was centrifuged (1500*g*, 10 min) at 4 °C using a refrigerated centrifuge (20PR-52D, Hitachi, Japan). Erythrocytes separated from the plasma and the buffy coat were washed three times by centrifugation (1500*g*, 5 min) in 10 vol. of 10 mM phosphate buffer saline (PBS), which consisted of 125 mM NaCl and 10 mM NaH_2PO_4 and Na_2HPO_4 in deionized water, adjusted to pH 7.4. The supernatant and buffy coat of white cells was carefully removed with each wash. During the last washing, the erythrocytes were obtained by centrifugation (1500*g*, 10 min). Washed erythrocytes were finally suspended using the same buffer to the desired hematocrit level and stored at 4 °C and used within 6 h of sample preparation.

A 0.1 ml of 10% suspension of erythrocytes was added to 0.2 ml of 200 mM AAPH and 0.1 ml of EPS with different concentrations (10–500 μg/ml). The reaction mixture was shaken gently, while being incubated at 37 °C for 3 h. The reaction mixture was diluted with 8 ml of PBS and centrifuged at 2000*g* for 10 min. The absorbance (A) of the resulting supernatant was measured at 540 nm by a spectrophotometer. Similarly, the reaction mixture was treated with 8 ml of distilled water to obtain complete hemolysis and the absorbance (B) of its supernatant in the same conditions was measured at 540 nm. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition } (\%) = (1 - A/B) \times 100 \quad (6)$$

Ascorbic acid was used as a positive control. Each test was performed at least three times and the results were reproducible within 10% deviation. Data represent the means ± SD of one representative experiment.

2.11. Statistical analysis

All analyses were performed in triplicate. The experimental results obtained were expressed as means ± SD. The EC_{50} value was estimated by a nonlinear regression algorithm (Data processing system, V3.01). Statistical analysis was performed using the software Statistica 6.0. Data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test.

3. Results and discussion

3.1. The extract from defatted peanut skins

Solvents used for extraction significantly affected the concentration of peanut skins extract at equal volume of solvent. The polar solvents were more efficient than less polar solvents (Huang et al., 2003), and ethanol and methanol were more effective in the extraction of phenolic compounds from peanut skins than water (Yu, Ahmedna, & Goktepe, 2005). In this study, the defatted peanut skins were extracted with aqueous ethanol (50%, v/v) and the yield of extraction was 0.107 ± 0.003 g dry matter of ethanolic extract per gram of defatted peanut skin. Huang et al. (2003) reported that the extraction with ethanol produced a yield of ethanolic extract of 0.051 g/g of peanut skin. Nepote, Grosso, and Guzman (2005) reported that the ethanol (96%, v/v) extracted 0.179 g soluble constituents from the peanut skins and 0.099 g from the defatted peanut skins. Duh et al. (1992) reported the yields of methanolic and ethanolic extracts from peanut hulls were 0.042 and 0.020 g/g of dried peanut hull, respectively. These results showed that peanut skins contained much more ethanol soluble constituents than hulls, and different yields of extract could be obtained using different extraction conditions.

3.2. Total phenolic content and total flavonoid content

Generally, the outer layers of plants such as the peel, shell, and hull contain large amounts of polyphenolic compounds to protect inner materials. Several hundreds

of different plant polyphenols have been identified (Scalbert et al., 2005). In the present work, the total phenolic content in EPS was determined by the Folin–Ciocalteu method, which is considered the best method for total phenolic content (including tannins) determination (Engelhardt, 2001). Total phenolic compounds of EPS were 0.909 ± 0.003 g per gram of extract, correspondingly, the one gram defatted peanut skins had 0.097 ± 0.003 g phenolic compounds. Lou et al. (2001) reported eight flavonoids were separated from the water soluble fraction of peanut skins. The total flavonoid content was also determined in the present study, which was 0.065 ± 0.003 g catechin equivalents/g of peanut skins tested. Nepote et al. (2005) reported that the phenolic content in peanut skins was 0.118 g/g dry peanut skin. Yu et al. (2005) reported that one gram dry peanut skin contained 0.090–0.125 g total phenolics, and the skin removal methods (such as direct peeling, blanching and roasting) and extraction solvents had significant effects on total extractable phenolics. It has also been reported that the contents of both luteolin and total phenolics of peanut hulls increased significantly with maturity (Yen & Duh, 1993) and the contents of both luteolin and total phenolic compounds of peanut hulls from various cultivars differed significantly (Yen & Duh, 1995).

3.3. DPPH radical scavenging activity

Phenolic compounds in plants are viewed as powerful in vitro antioxidants due to their ability to donate hydrogen or electrons and to form stable radical intermediates (Scalbert et al., 2005). DPPH, a stable nitrogen centered free radical, has been used to evaluate natural antioxidants for their radical quenching capacities in a relatively short time compared with other methods. The method is based on the reduction of the absorbance of methanolic DPPH solution at 517 nm in the presence of a proton-donating substance, due to the formation of the diamagnetic molecule by accepting an electron or hydrogen radical (Soares, Dins, Cunha, & Ameida, 1997). To better understand its antioxidant mechanism(s), EPS was evaluated for radical scavenging activities against DPPH. As shown in Fig. 1, EPS exhibited a strong scavenging activity on the DPPH radical in vitro assay. A significant ($p < 0.05$) increase of the scavenging activity was observed at the concentration range (0–100 μg/ml) of EPS, and the scavenging reached a maximum plateau at round 97% from 10 to 500 μg/ml. The estimated EC_{50} value of EPS (30.8 μg/ml), which stands for the concentration of an antioxidant required to scavenge 50% of the radicals in the reaction mixture under the experimental conditions, was lower than that of the synthetic antioxidant, BHA (40.0 μg/ml).

3.4. Chelating activity

It has been recognized that transition metals are involved in both initiation and propagation of lipid peroxidation (Hsiao, Teng, Wu, & Ko, 1996). Undoubtedly, the

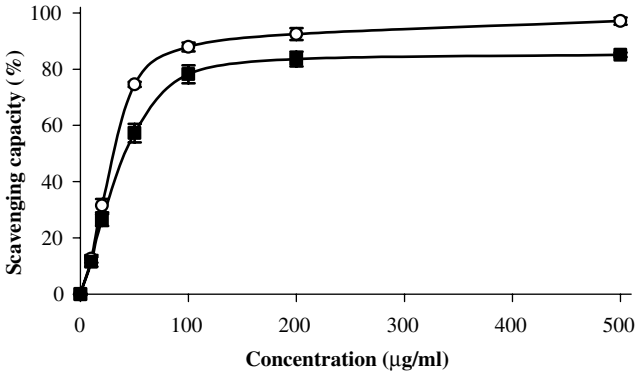

Fig. 1. Scavenging capacity of DPPH radical of EPS (○) and BHA (■) at different concentrations (10–500 μg/ml). Data represent the means ± SD ($n = 3$).

compounds interfering with the catalytic activity of metal ions could affect the peroxidative process. Flavonoids were known to retain free radical scavenging capacity by forming less reactive flavonoid phenoxyl radicals and chelating transition metal ions (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Fe^{2+} ion is the most powerful prooxidant among various species of metal ions (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Thus, Fe^{2+}-binding activity of EPS was determined by 2,2′-bipyridyl competition assay. 2,2′-Bipyridyl can quantitatively form red complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Therefore, the chelating effect of the coexisting chelator was estimated by measurement of the rate of red color reduction (Yamaguchi et al., 2000). In the assay, EPS interfered with the formation of ferrous and bipyridyl complex, suggesting that they have chelating activity and capture ferrous ions before bipyridyl. The Fe^{2+}-chelating effect of EPS at various concentrations (10–500 μg/ml) is shown in Fig. 2. The ferrous ion chelating effect of EPS was significantly ($p < 0.05$) increased at the concentration range (10–100 μg/ml). Although EPS had an overall lower chelating effect than

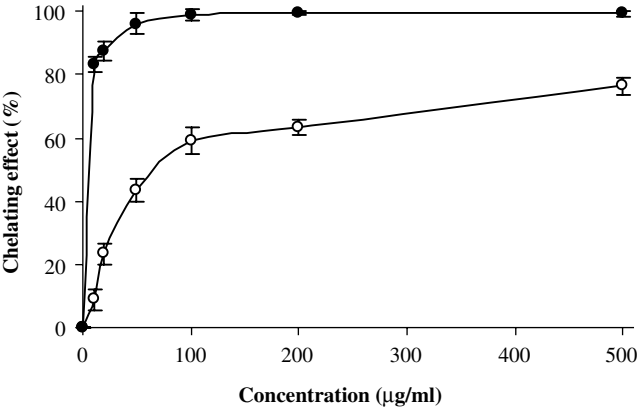

Fig. 2. Chelating effect of EPS (○) and EDTA (●) at different concentrations (10–500 μg/ml) on Fe^{2+}. Data represent the means ± SD ($n = 3$).

EDTA, 76.6% maximum effect was evident at 500 μg/ml, which was comparable to that of the chelating activity of EDTA at the lowest concentration of 10 μg/ml. Significant Fe^{2+}-chelating capacity of EPS demonstrated that it might reduce the concentration of the catalysing transition metal in lipid peroxidation and suppress the superoxide-driven Fenton reaction (Eqs. (7) and (8)), which is currently believed to be the most important route to active oxygen species (Arora, Nair, & Strasburg, 1998), thereby, protecting against oxidative damage

$$O_2^{\cdot-} + Fe^{3+} \rightarrow O_2 + Fe^{2+} \quad (7)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^- \quad (8)$$

3.5. Hydroxyl radical scavenging activity

Among the oxygen radicals, hydroxyl radicals are the most reactive and induce severe damage to the adjacent biomolecules (Chance, Sies, & Boveris, 1979). Competition between deoxyribose and the sample against hydroxyl radical generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system was measured for determination of hydroxyl radical scavenging activity in this study. Fig. 3 showed the ability of EPS at different concentrations (10–500 μg/ml) to scavenge hydroxyl radical compared with BHA as a standard. EPS scavenged hydroxyl radical in a concentration dependent way. The hydroxyl radical scavenging capacity of EPS was significant at all the tested concentrations. At 500 μg/ml, EPS exhibited 85.3% of scavenging activity on hydroxyl radical, however, BHA exhibited 53.3% of scavenging activity on hydroxyl radicals. The estimated EC_{50} value of EPS (84.0 μg/ml) was 4 times ca. lower than that of the synthetic antioxidant, BHA (389 μg/ml). Generally molecules that inhibit deoxyribose degradation are those that can chelate the iron ions and thereby prevent them from complexing with the deoxyribose and render them inactive in a Fenton reaction (Smith, Halliwell, & Aruoma, 1992). As shown in Fig. 2, EPS exerted marked Fe^{2+} chelating activity, suggesting that EPS minimize the concentration of Fe^{2+}in the Fenton reaction. Overall, the scavenging activities of EPS might be due to the active hydrogen donating ability of hydroxyl substitutions of phenolic compounds presented in EPS as phenolic compounds are good electron donors, they may accelerate the conversion of H_2O_2 into H_2O.

3.6. Superoxide anion scavenging activity

Superoxide anion is produced by a number of enzyme systems in autooxidation reactions and by nonenzymatic electron transfers that univalently reduce molecular oxygen. In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified because it produces other types of cell-damaging free radicals and oxidizing agents, which has been implicated in the initiating oxidation reactions associated with aging (Liu & Ng, 2000).

It has been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radicals (Robak & Gryglewski, 1988). In the present study, the superoxide anion radical scavenging activity of EPS was determined by the xanthine–xanthine oxidase system. The decline of absorbance at 560 nm reflected the elimination of superoxide anion radical. Fig. 4 showed the percentage inhibition of superoxide anion radicals by EPS and BHA at different concentrations (10–500 μg/ml). EPS scavenged superoxide anions in a concentration dependent manner. Significant ($p < 0.01$) scavenging (34.8–98.6%) of superoxide radicals was evident at the all tested concentrations of EPS (10–500 μg/ml). The estimated EC_{50} value of EPS (25.4 μg/ml) was 5 times lower than that of the synthetic antioxidant, BHA (152.1 μg/ml). Furthermore, these results revealed that EPS was apparently more potent than the nonenzymatic superoxide scavenger BHA. Therefore, EPS might be possibly advantageous in the micromolar range for preventing injury induced by superoxide anion radicals in pathological conditions.

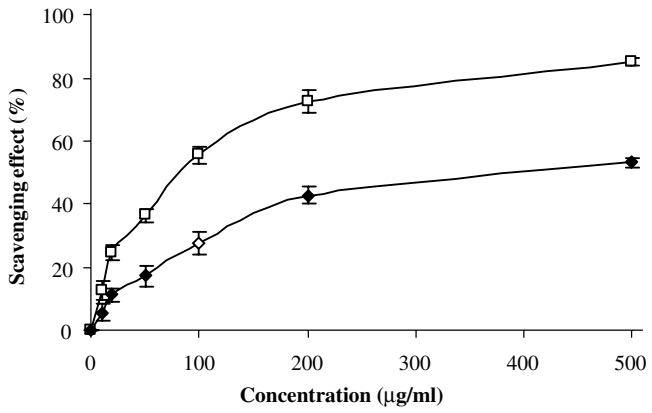

Fig. 3. Scavenging effect of EPS (□) and BHA (◆) at different concentrations (10–500 μg/ml) on hydroxyl radical. Data represent the means ± SD ($n = 3$).

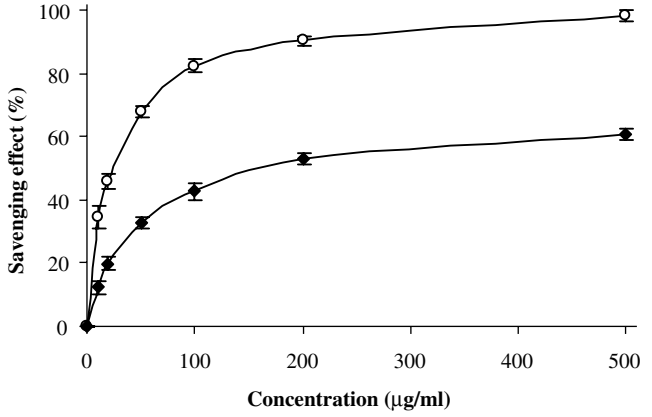

Fig. 4. Scavenging effect of EPS (○) and BHA (◆) at different concentrations (10–500 μg/ml) on superoxide anion radical. Data represent the means ± SD ($n = 3$).

3.7. Hydrogen peroxide scavenging activity

Hydrogen peroxide can be formed in vivo by many oxidizing enzymes such as superoxide dismutase. Hydrogen peroxide together with reactive oxygen species (ROS) can damage several cellular components. Hydrogen peroxide is a relatively unstable metabolic product being responsible for the generation of hydroxyl radicals and singlet oxygen, which is formed by Fenton reaction and initiate lipid peroxidation or are toxic to cells. Thus, the removing of hydrogen peroxide is very important for antioxidant defence in cells. Fig. 5 depicted the hydrogen peroxide scavenging activity of EPS and compared with BHA as standard. EPS were capable of scavenging hydrogen peroxide in an dose dependent manner. Significant ($p < 0.01$) hydrogen peroxide scavenging activity was obvious at all the tested concentrations of EPS (10–500 μg/ml). At 500 μg/ml, EPS exhibited 89.1% of scavenging activity on hydrogen peroxide, however, BHA exhibited only 37.3% of scavenging activity on hydrogen peroxide. These results showed that EPS had stronger hydrogen peroxide scavenging activity. Scavenging of hydrogen peroxide by EPS might be attributed to the phenolics in it, which could donate electrons to hydrogen peroxide, thus neutralizing it to water, as shown in the following:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad (9)$$

3.8. Inhibition of normal human erythrocyte hemolysis induced by peroxyl radical

Erythrocytes have been used as a model to investigate oxidative damage in biomembranes due to their high susceptibility to peroxidation (Liu & Ng, 2000). In this study, the protection of oxidative damage by EPS in normal human red blood cells was investigated. The erythrocyte hemolysis was induced by AAPH, a water soluble azo compound, which is a peroxyl radical initiator that generates free radicals by its decomposition at physiological temperature at a constant rate in a water environment without biotransformations or enzymes (Miki, Tamai, Mino, Yamamoto, & Niki, 1987). Fig. 6 shows the inhibitory effect of different concentrations of EPS (10–500 μg/ml) on AAPH-induced hemolysis of 10% human erythrocytes for 3 h, as a result of protection against the oxidative damage to cell membranes of human erythrocytes. A significant ($p < 0.01$) increase of inhibition percentage of hemolysis was observed at the concentration range (0–100 μg/ml) of EPS. EPS showed a significantly ($p < 0.05$) higher inhibition percentage of hemolysis (22.8–98.6%) than ascorbic acid (8.7–84.4%) at the concentration range of 10–500 μg/ml and inhibited hemolysis of erythrocytes in a dose-dependent manner. The estimated EC_{50} value of EPS (29.4 μg/ml) was much lower than that of ascorbic acid (88.1 μg/ml). These results indicated that the water-soluble EPS had a greater protective effect against hemolysis of red blood cells than ascorbic acid under in vitro conditions.

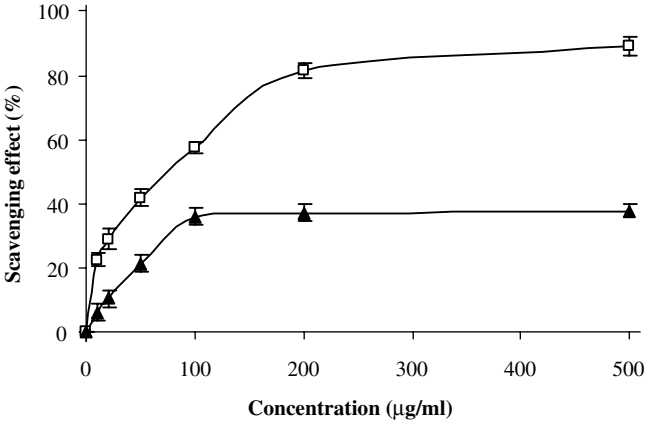

Fig. 5. Scavenging effect of EPS (□) and BHA (▲) at different concentrations (10–500 μg/ml) on hydrogen peroxide. Data represent the means ± SD ($n = 3$).

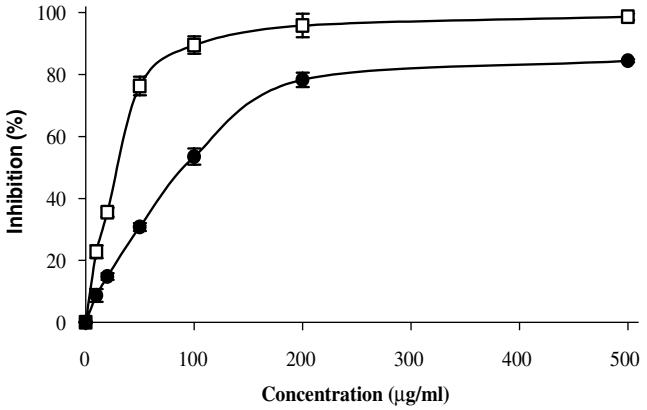

Fig. 6. Inhibitory effect of EPS (□) and ascorbic acid (●) at different concentrations (10–500 μg/ml) on hemolysis of 10% human erythrocytes induced by 200 mM AAPH for 3 h at 37 °C. Data represent the means ± SD ($n = 3$) of one representative experiment.

The membrane of erythrocytes is rich in polyunsaturated fatty acids that are very susceptible to free radical mediated peroxidation. The peroxyl radicals generated from AAPH can attack the polyunsaturated fatty acids in the membrane of erythrocytes to induce the chain oxidation of lipid and protein, disturbing the membrane organization and eventually leading to hemolysis. The inhibitory effect of EPS on the oxidative hemolysis of human erythrocyte might be related to their scavenging activity on peroxyl radicals, thereby inhibiting the peroxidation of erythrocytes in vitro.

4. Conclusions

The water soluble extract from defatted peanut skins contains a number of phenolic compounds including flavonoids, which can effectively scavenge DPPH radical and various reactive oxygen species, chelate Fe^{2+}, inhibit the

hemolysis of normal human erythrocyte induced by peroxyl radicals in vitro conditions, suggesting that multiple mechanisms are responsible for the antioxidant activity of EPS. The significant antioxidant activity of EPS demonstrated that it might alleviate oxidative damage in cell induced by oxygen radicals, thereby, being beneficial to human health and effectively employed as an ingredient in food applications. The in vivo antioxidant activity of EPS and the isolation of the antioxidative components will be further investigated.

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