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Antioxidant Activity of a Flavonoid-Rich Extract of *Hypericum perforatum* L. in Vitro

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A flavonoid-rich extract of *Hypericum perforatum* L. (FEHP) was prepared by adsorption on macroporous resin and desorption by ethanol. Total flavonoid content of FEHP was determined by a colorimetric method. The major constituents of FEHP, including rutin, hyperoside, isoquercitrin, avicularin, quercitrin, and quercetin, were determined by HPLC analysis and confirmed by LC-MS. Different antioxidant assays were utilized to evaluate free radical scavenging activity and antioxidant activity of FEHP. FEHP was an effective scavenger in quenching DPPH and superoxide radical with IC_{50} of 10.63 μg/mL and 54.3 μg/mL, respectively. A linear correlation between concentration of FEHP and reducing power was observed with a coefficient of $r^2 = 0.9991$. Addition of 150 μg of FEHP obviously decreased the peroxidation of linoleic acid during 84 h incubation, but the amount of FEHP over 150 μg did not show statistically significant inhibitory effect of peroxidation of linoliec acid ($p > 0.05$). FEHP exhibited inhibitory effect of peroxidation of liposome induced both by hydroxyl radical generated with iron-ascorbic acid system and peroxyl radical and showed prominent inhibitory effect of deoxyribose degradation in a concentration-dependent manner in site-specific assay but poor effect in non-site-specific assay, which suggested that chelation of metal ion was the main antioxidant action. According to the results obtained in the present study, the antioxidant mechanism of FEHP might be attributed to its free radical scavenging activity, metal-chelation activity, and reactive oxygen quenching activity.

KEYWORDS: ***Hypericum perforatum* L.; antioxidant activity; flavonoid; free radicals; reducing power; lipid peroxidation; liposome; deoxyribose degradation**

INTRODUCTION

Free radicals, having one or more unpaired electrons in the outer orbit, include superoxide anion ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and nitric oxide, which are oxygen-centered free radicals, sometimes known as reactive oxygen species (ROS) (*1*, *2*). Modern theories of ROS have revealed that ROS play a dual role in organisms. ROS are not only strongly associated with lipid peroxidation, which lead to the deterioration of the food, but also involved in the development of a variety of diseases including cellar aging, mutagenesis, carcinogenesis, coronary heart disease, diabetes, and neurodegeneration (*3*−*5*).

More and more studies have shown that antioxidant from plant can be correlated with oxidative stress and age-dependent diseases (*6*). Flavonoid, abundant in fruits, teas, vegetables, and medicinal plants, have received the greatest attention and have been investigated extensively, since they are highly effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants such as BHA and BHT, which are suspected of being carcinogenic and causing liver damage (*7*, *8*).

Hypericum perforatum L.(HPL), known as St John's Wort, is a perennial herbaceous plant of the Hypericaceae family and is distributed in Europe, Northern Africa, Northern America, and the Shandong, Hebei, and Guizhou provinces in China (*9*, *10*). HPL has been used traditionally for the treatment for mild to moderate depression, and clinical studies have suggested that extract of HPL is as effective as traditional antidepressant and has superior efficacy compared to placebo (*11*, *12*). However, the antidepressive mechanism of HPL is unclear and controversial; more than 10 components have been found in HPL, including flavonoids, phloroglucinols and naphthodiathrones. Flavonol derivatives such as quercitrin, rutin, and astilbin, naphthodiathrones such as hypericin and pesudohypericin, and phloroglucinols such as hyperforin and adhyperforin showed antidepressant activity in different antideperessive model systems (*13*−*17*). Moreover, two recent clinical studies have showed that HPL was ineffective in the treatment for moderately severe major depression (*18*, *19*). Flavonoid is rich in HPL, in which the content is 11.71% in flowers and 7.4% in leaves and stems (*20*). But so far, the total flavonoid from HPL are not

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fully studied, and no studies have been conducted to investigate the antioxidant activity of this medicinal plant.

The antioxidant activity of plant extract cannot be evaluated by only a single method due to the complex nature of phytochemicals, so it is important to employ commonly accepted assays to evaluate the antioxidant activity of plant extract. Numerous antioxidant methods have been developed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, superoxide anion scavenging, and hydroxyl radical scavenging assay are most commonly accepted assays to evaluate antioxidant activity (*21*, *22*). In view of this, a flavonoid-rich extract of HPL (FEHP) was prepared by adsorption on macroporous resin and desorption by ethanol, and its antioxidant activity was evaluated by various tests in the present study.

MATERIALS AND METHODS

Materials and Chemicals The raw extract of HPL (REHP) was a gift from Tianjin Jianfeng Natural Product R&D Co. Ltd (Tianjin, China) and the content of hypericin was 0.3%, as marked by the label. Rutin, hyperoside, isoquercitrin, and quercetin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Quercitrin and avicularin were isolated as previously reported (*23*), the purity (>96%) was confirmed by HPLC. Tea polyphenol (>98%) was a product of Wuxi Lvbao Natural Additives Co. Ltd. (Wuxi, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), linoleic acid (98%), α-tocopherol, 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 2-deoxy-D-ribose, phenazin methosulfate (PMS), reduced β-nicotinamide adenine dinucleotide (NADH), and nitroblue tetrazolium (NBT) were purchased from Fluka Biochemika AG (Switzerland), tertiary butyhydroquinone (TBHQ) was purchased from ACROS ORGANICS (New Jersy), all other chemicals were of analytical grade.

Preparation of FEHP. FEHP was prepared by the following steps: The REHP was suspended in water by ultrasonication, then the solution was centrifuged in an Eppendorf 5840R centrifuge at 3000 rpm for 10 min to remove the unsoluble and the supernatant was filtrated through Whatman No 1 filter paper by vacuum sucking, to obtain a clarified solution. The solution was poured in a column previously packed with a nonionic polystyrene resin LSA (Lanshen Exchange and Adsorption Resin Ltd., Xi'an, China) (column of 40 × 2.6 cm, i. d.). The solution was pumped down through the column at a speed of 2.25 bed volumes/h (BV/h). When the content of total flavonoid in the effluent achieved a value of about 10% of the loaded solution, as measured by the colorimetric method, the resin was thought to be saturated and the loading was then stopped. The resin was washed with 5 BV distilled water to remove the sugars and other water-soluble compounds, then 75% ethanol was used to elute the flavonoid at a speed of 4.5 BV/h. The effluent between 0.25 and 3BV was collected, and the solvent was removed with a rotary evaporator at 70 °C to afford a final extract (FEHP).

Determination of Total Flavonoid Content. The total flavonoid content of REHP and FEHP was determined by use of a slightly modified colorimetric method described previously (*24*). A 0.5-mL aliquot of appropriately diluted sample solution was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5% $NaNO_2$ solution. After 6 min, 0.15 mL of a 10% $AlCl_3$ solution was added and allowed to stand for 6 min, then 2 mL of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 mL, then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. All values were expressed as milligrams of rutin equiv per 1 gram of REHP. Data were reported as means ± SD for three replications.

High-Performance Liquid Chromatography (HPLC) Analysis. The constituents of FEHP were analyzed by an Agilent 1100 series system, equipped with a phtodiode array detector working in the range of 190∼810 nm, a quaternary pump, and an autosampler. The absorption was set at 360 nm for most constituents. The chromatographic data were recorded and processed with Agilent Chromatographic Work Station software. Analysis was carried out at 40 °C on an Agilent Eclipse XDB−C 18 column (3.5 μm, 150 × 4.6 mm, i. d.), which was protected by a guard column (3.5 μm, 12.5 × 4.6 mm, i. d.). The mobile phase consisted of methanol (A), acetonitrile (B), and 20 mM ammonium acetate buffer adjusted to pH 3.5 with glacial acetic acid (C). The analysis was performed by use of a linear gradient program. Initial conditions were 1.3% A changed to 1.7%, 11.7% B changed to 15.3% in 10 min, and in another 25 min, changed to to 10% A and 90% B. Each run was followed by an equilibration period of 20 min. The flow rate was kept at 1.0 mL/min, and the injection volume was 5 μL.

LC-MS was performed on an HP 1100 mass spectrometer equipped with an HP 1100 HPLC system. The mass spectrometer was operated in negative electrospray mode to obtain the best result, with the ionization voltage of 70 V, source voltage of 3.5 KV. Nitrogen was used as nebulizing gas at 40 psi and drying gas at 350 °C. Full scans were acquired from *m*/*z* 50−1000 at 1.2 scans/s. Component separation was carried out using the same conditions in HPLC analysis, except that the flow rate was reduced to 0.8 mL/min.

Determination of Scavenging Activity on DPPH. The radical scavenging activity of FEHP against DPPH free radical was measured using the method of Brand-Williams et al. (*25*), slightly modified as follows: 1.5 mL of ethanolic solution of DPPH (2×10^{-4} mol/L) was mixed with equivalent aliquot of 20 μg/mL antioxidant solutions in a 1-cm cuvette. Absorbance at 517 nm was measured at 2-min intervals by use of a UV-7504 spectrophotometer (Shanghai, China) until the absorbance reached a plateau. To determination of scavenging effect of different concentration compounds on DPPH, the same method was used, except that 20 μg/mL antioxidant solutions was replaced by 1.5 mL of different concentrations of samples, and after standing in dark for 30 min, absorbance at 517 nm was measured against ethanol. Controls containing ethanol instead of the antioxidant solution and blanks containing ethanol instead of DPPH solution were also made. The inhibition of the DPPH radical by the samples was calculated according to the following formula: DPPH scavenging activity (%)= (1 − (Abs. of sample-Abs. of blank)/Abs. of control) × 100. The percentage of scavenging activity was plotted against the sample concentration to obtain the IC_{50}, defined as the concentration of sample necessary to cause 50% inhibition.

Determination of Antioxidant Activity in a Linoleic Acid System. The total antioxidant activity of FEHP was carried out by use of a linoleic acid system (*26*). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the mixture was homogenized. A 0.5-mL ethanol solution of different concentration of FEHP (50−500 μg/mL) was mixed with linoleic acid emulsion (2.5 mL, 0.2 M, pH 7.0) and phosphate buffer (2 mL, 0.2 M, pH 7.0). The reaction mixture was incubated at 37 °C in the dark to accelerate the peroxidation process. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 20 mM in 3.5% HCl). After the mixture was left for 3 min, the peroxide value was determined by reading the absorbance at 500 nm on a spectrophotometer.

Determination of Reducing Power. The reducing power of the prepared extracts was determined according to the method of Oyaizu (*27*). Briefly, 1.0 mL of different concentration sample (12.5−400 μg/mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min. Afterward, 2.5 mL of a 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 3000 rpm for 10 min. A 2.5-mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of a 0.1% (w/v) solution of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. Increased absorbance of the reaction mixture indicates greater reducing power.

Determination of Scavenging Activity on Superoxide Anion. Superoxide anion radicals are generated in a PMS−NADH system by oxidation of NADH and assayed by the reduction of NBT (*28*). In this

experiment, the superoxide anion radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 μM NADH, 50 μM NBT, 10 μM PMS, and samples at different concentrations (initial concentrations were from 15.63 to 500 μg/mL). PMS was finally added to initiate the reaction; after 5 min incubation at room temperature, the color reaction between superoxide anion radical and NBT was read at 560 nm spectrophotometrically. Mixture without sample was used as control and mixture without PMS was used as blank. The scavenging activity was calculated as follows: scavenging activity (%) = (1 − (Abs. of sample-Abs. of blank)/Abs. of control) × 100, and the IC_{50} was calculated according to the relationship of concentration and scavenging activity.

Determination of Antioxidant Effect on Liposome Peroxidation. Liposomes were prepared according to the method of Duch P. D. (*29*), and the effect on liposome peroxidation was assayed by the method of Yen G. C. (*30*) with slight modification. In brief, 400 mg of soybean lecithin was dispersed in 40 mL of phosphate buffer (0.2 M, pH 7.4) and sonicated in a sonicator for 2 h under ice-water bath to obtain a milk-white solution. The peroxidation was initiated either by ascorbic acid/ferric chloride redox system or AAPH, a water-soluble peroxyl radical initiator. A typical peroxidation reaction contained 25 mM $FeCl_3$, 25 mM H_2O_2, 5 mg liposome, 25 mM ascorbic acid, solutions of FEHP at different concentration, and phosphate buffer (0.2 M, pH 7.4) to make the final volume 2 mL. Alternatively, peroxidation was initiated by the addition of a hydrophilic free radical initiator AAPH at a final concentration of 10 mM. The reaction mixture was incubated at 37 °C for 4 h. At the end of incubation, 1 mL of BHT (2% in ethanol) was added to the mixture to stop the oxidation reaction. The extent of oxidation was subsequently determined by measuring the thiobarbituric acid reactive substance (TBARS). The mixture was added with 0.5 mL of 1% TBA (in 50 mM NaOH) and 0.5 mL of 10% HCl, then it was heated in a water bath at 90 °C for 20 min. After cooling, 2.5 mL of *n*-butyl alcohol was added, and the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents except lecithin. Inhibition percent of liposome peroxidation was calculated as 100 × (1 − Abs. of sample/Abs. of control).

Determination of Inhibitory Effect on Deoxyribose Degradation. Inhibitory effect of FEHP on deoxyribose degradation was determined by measuring the competition between deoxyribose and FEHP for the hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (referred to nonsite-specific assay) or Fe^{3+}/ascorbate/H_2O_2 system (referred to site-specific assay). The attack of the hydroxyl radical on deoxyribose leads to TBARS formation (*31*). Solutions of the reagents were made up in deaerated water immediately before use. The test sample of different concentration was added to the reaction mixture containing 3.0 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2, and 20 mM phosphate buffer (pH 7.4) and made up to a final volume of 1.2 mL. The amount of TBARS formed following 1 h of incubation at 37 °C was measured according to the method of Ohkawa et al. (*32*). A 1.0-mL aliquot of thiobarbituric acid (TBA, 1%) and 1.0 mL of trichloroacetic acid (TCA, 2.8%) were mixed with the reaction mixtures in the tube, and the mixtures were then incubated at 100 °C for 20 min. After the mixtures were cooled to room temperature, their absorbances at 532 nm were measured against a blank containing deoxyribose and buffer. For site-specific hydroxyl radical scavenging activity, the procedure was similar to the above method, except that EDTA was replaced by the equivalent volume of buffer (*33*). Percent inhibition (PI) of deoxyribose degradation was calculated with the equation IP (%) = (1 − Abs. of tested sample/Abs. of control) × 100.

Statistical Analysis. The data were expressed as the mean of three replicate determinations and standard deviation (SD), statistical comparisons were made with student's test. P values of < 0.05 were considered to be significant, and p values of < 0.01 were considered to be highly significant.

RESULTS AND DISSCUSIONS

The Yield, Total Flavnoid Content, and Composition of FEHP. The macroporous adsorption resin has been extensively used to recover flavonoid from plant extract with the removal of other soluble compounds (*34*). There was a significant difference in total flavonoid content of REHP and FEHP, in which the content was 285.6 ± 3.7 mg/g and 861.0 ± 4.8 mg/g, respectively. After purified by LSA resin, FEHP presented approximately three times flavonoid content than REHP, and the yield of FEHP was 22.03% based on REHP. The HPLC analysis of REHP and FEHP revealed that flavonol such as quercetin and its glucoside were presented in both REHP and FEHP. The HPLC profiles of REHP and FEHP were shown in **Figure 1**. The main compounds, rutin (**1**), hyperoside (**2**), isoquercitrin (**3**), avicularin (**4**), quercitrin (**5**), and quercetin (**6**), were identified by comparison of their relative retention time with authentic standards and confirmed by LC-MS. The proportion of major flavonoid was 3.72, 14.89, 5.04, 10.83, 6.31, and 50.07% and 7.21, 22.56, 8.04, 18.40, 13.86, and 15.11% in REHP and FEHP, respectively. The LC-ESI-MS data and structure for components of FEHP are shown in **Table 1**. Most flavonoids were enriched in FEHP; however, content of quercetin of FEHP was lower than that of REHP, the reason might lie in that the polarity and solubility in water of quercetin are less than its glucoside such as rutin and hyperoside, so the adsorption capacity on resin decreased and the content of quercetin in FEHP was lower than that in REHP.

DPPH Scavenging Activity. The DPPH radical is a stable organic free radical with adsorption band at 515−528 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. Because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentrations, it has been extensively used for screening antiradical activities of fruit and vegetable juices or extracts (*22*). The results in **Figure 2A** demonstrated that after 30-min incubation, the absorbance of reaction mixture did not change over the period of the experiments in the presence or absence of test samples, which suggested that 30 min of reaction time was enough to determine the antioxidant activity of various samples in the model. **Figure 2B** illustrated the scavenging activity of FEHP and some pure compounds found in FEHP. All samples showed a dose-dependent manner in scavenging DPPH radical that was statistically significant ($p < 0.05$) compared with control. The IC_{50} of FEHP, hyperoside, rutin, quercetin, TBHQ, and α-tocopherol was 10.63, 9.98, 15.25, 5.02, 8.32, and 22.19 μg/mL, respectively. Quercetin possessing 5 hydroxy was the most effective antioxidant in scavenging DPPH, but presence of a sugar residue resulted in a decrease of antioxidant activity, as was found for rutin and hyperoside. Although it seemed that there were no synergistic effect for FEHP since IC_{50} of FEHP was lower than that of quercetin and equal to hyperoside, two primary components in FEHP, its antioxidant activity in scavenging DPPH overmatched α-tocopherol.

Antioxidant Activity in Linoleic Acid System. In the present study, the antioxidant activity of FEHP, determined by peroxidation of linoleic acid using the thiocyanate method at 37 °C, after addition of different concentrations of FEHP, was determined. During the linoleic acid peroxidation, peroxides are formed and these compounds oxidize Fe^{2+} to Fe^{3+}, the latter Fe^{3+} ion forms a complex with SCN^-, which has a maximum absorbance at 500 nm. High absorbance is an indication of high concentration of peroxide formed during the emulsion incubation.

The antioxidant activity of FEHP exhibited an amount-dependent manner, as can be seen from **Figure 3**. In the control

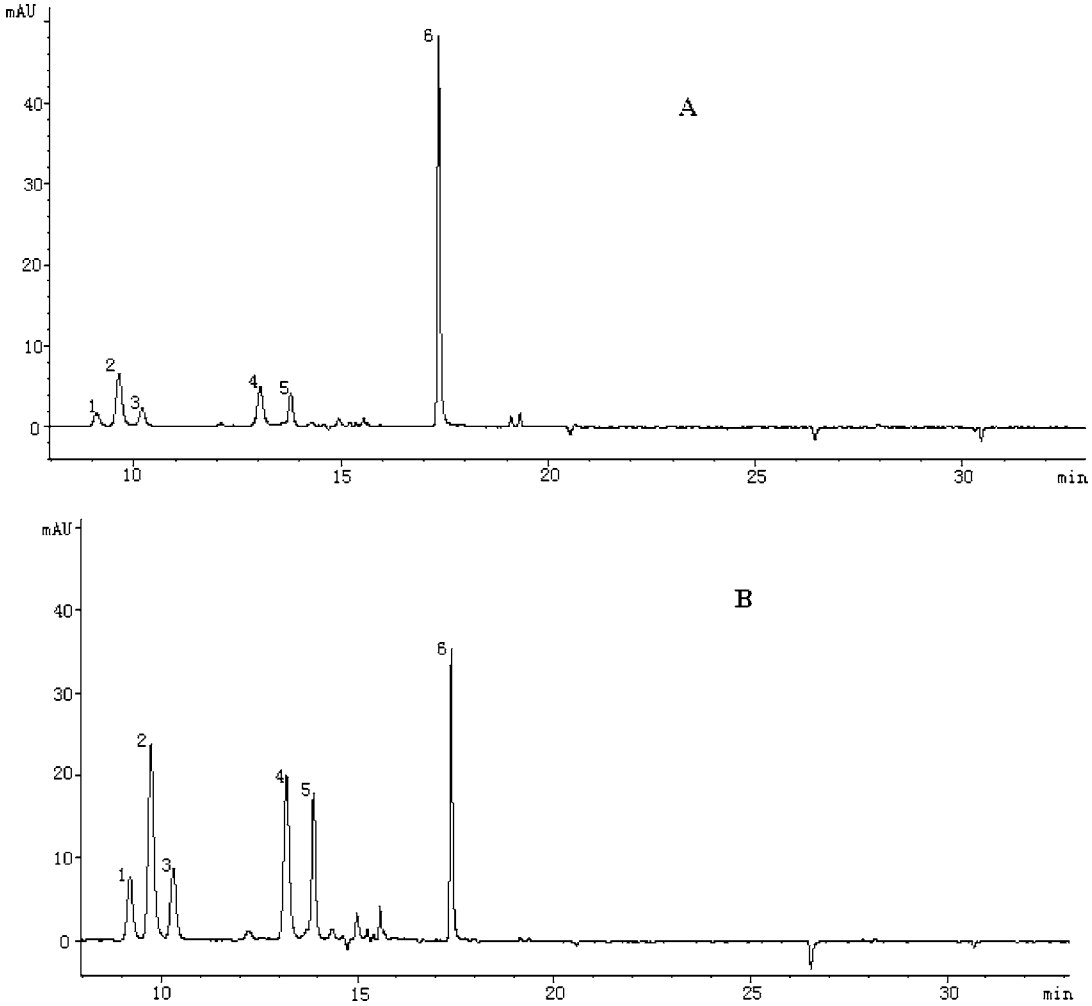


Figure 1. HPLC profiles of REHP (**A**) and FEHP (**B**) recorded at 360 nm. Peak identifications: (1) rutin; (2) hyperoside; (3) isoquercitrin; (4) avicularin; (5) quercitrin; (6) quercetin.

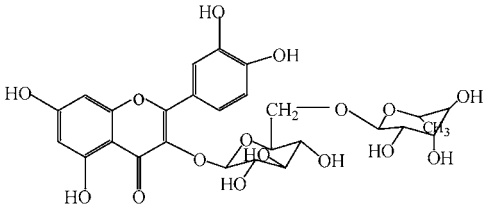
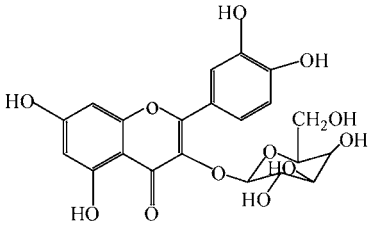
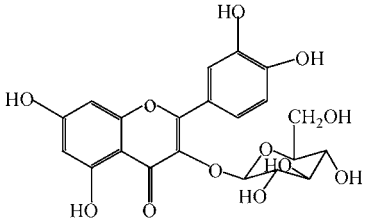
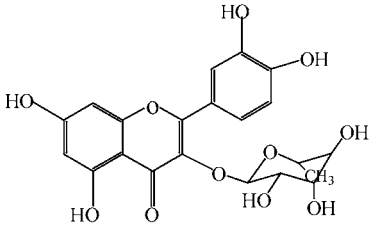
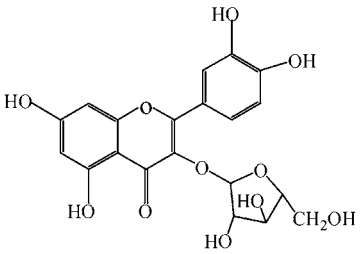
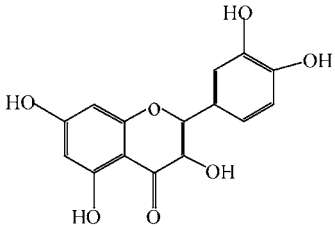
without FEHP, the absorbance at 500 nm increased up to a maximal value of 1.985 ± 0.143 at 84 h, then it decreased. The reason was that linoleic acid hydroperoxides, generated from the peroxidation of linoleic acid, decomposed to many secondary oxidation products, or the intermediate products may be converted to stable end-products and the substrate was exhausted (*35*). Of the six tested concentrations, even addition of 25 μg of FEHP into the emulsion was able to reduce the formation of hydroperoxide. Although the antioxidant activity of FEHP increased with increasing amount of FEHP, there was no statistically significant difference between 150, 200, and 250 μg of FEHP.

Reducing Power. The reducing power of FEHP, which may sever as a significant reflection of the antioxidant activity, was determined using a modified iron (III) to iron (II) reduction assay. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of extracts or compounds. The presence of reductants in the solution causes the reduction of the Fe^{3+}/Ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700 nm.

Figure 4 showed the reducing power of FEHP compared to TBHQ, quercetin, rutin, hyperoside, ascorbic acid, and tea polyphenol. All samples showed some degree of reducing power; however, as anticipated, their reducing power was inferior to ascorbic acid, which is known to be a strong reducing agent. Like the antioxidant activity, the reducing power of FEHP increased with increasing amount of FEHP; the equation of reducing power (y) and amount of FEHP (x) was $y = 0.048x + 0.2401$ ($r^2 = 0.9991$), indicating that reducing ability correlated well with amount of FEHP. The reducing power of FEHP and other compounds followed the following order: ascorbic acid > tea polyphenol > quercetin > TBHQ > hyperoside > FEHP > rutin.

Superoxide Anion Scavenging Activity. In the PMS−NADH−NBT system, superoxide anion derived from dissolved oxygen by PMS−NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. **Figure 5** showed the superoxide anion radical scavenging activity of different concentrations of FEHP, tea polyphenol, hyperoside, rutin, and quercetin. Both of them had strong superoxide radical scavenging activity in a dose-dependent manner, but tea polyphenol, used as a positive control, exhibited the highest superoxide radical scavenging activity. The results were found statistically significant ($p < 0.05$) with the control. IC_{50} of FEHP, tea polyphenol, hyperoside, rutin, and quercetin was 54.3, 16.7, 28.3, 39.8 and 136.5 μg/mL, respectively. Superoxide anion scavenging activity of those samples followed the following order: tea polyphenol > hyperoside > rutin > FEHP > quercetin. Although quercetin had the most electron-donating capacity and hydrogen-donating capacity in reducing

Table 1. Structure and Ions in LC-ESI-MS for Confirmation of Compounds in FEHP

Compound	m/z	Structure
Rutin	301,463,609	
Hyperoside	301,463	
Isoquercitrin	301,463	
Quercitrin	301,447	
Avicularin	301,433	
Quercetin	301	

iron (III) to iron (II) and quenching DPPH, its scavenging effect on superoxide anion was the weakest.

Inhibition of Liposome Peroxidation. Phospholipids are believed to be present in high amounts in cell membranes. To investigate FEHP in a biological system, the phospholipid prepared as a liposome was used to evaluate the effect of FEHP on liposome peroxidation. FEHP inhibited liposome peroxidantion as assayed by the amount of TBARS. The efficacy of FEHP to prevent the peroxidation of soybean lecithin liposome, induced by hydroxyl radical generated with Fenton chemical reaction or by peroxyl radical generated with AAPH, was present in **Figure 6**. FEHP exhibited about 6.34−70.0% inhibition of liposome peroxidation in the range of 50−800 μg/mL, and the inhibition effect was similar to that of α-tocopherol.

In this study, peroxidation induced by peroxyl radical was also investigated. The inhibition effect was also amount-

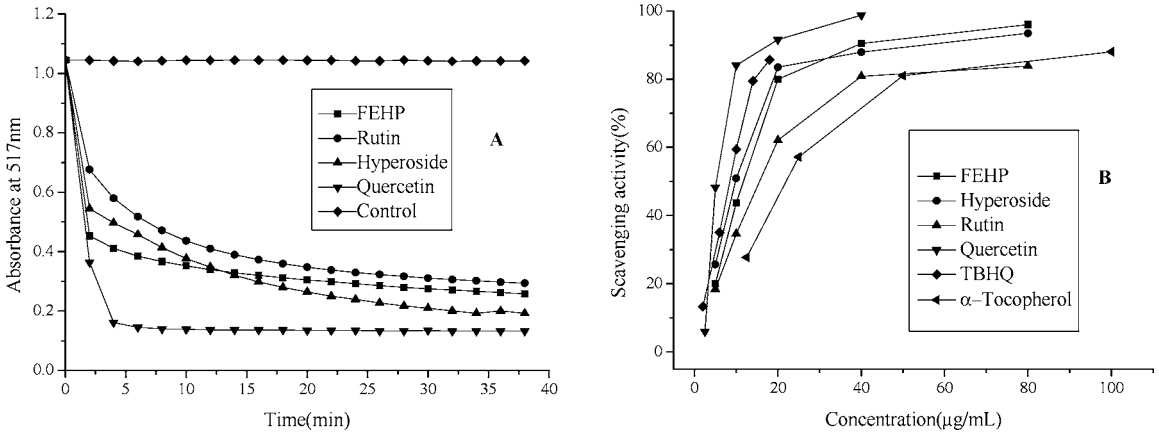


Figure 2. Kinetics curve of scavenging DPPH radical (**A**) and scavenging effect on DPPH (**B**) by FEHP and some pure compound found in *Hypericum perforatum* L.

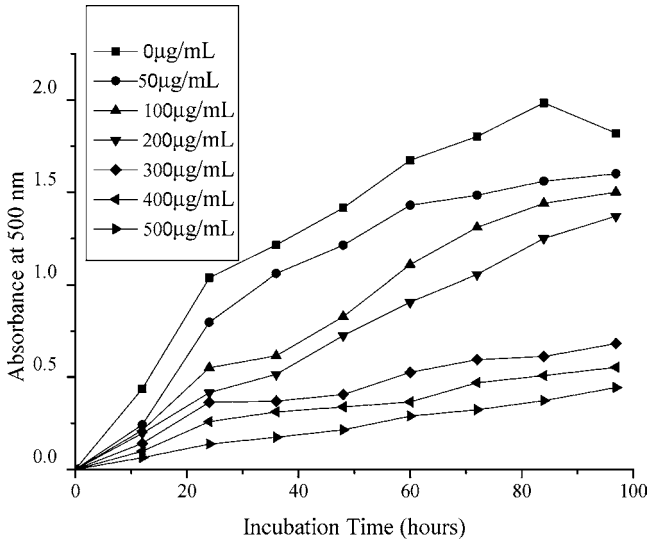


Figure 3. Antioxidant activity of different doses of FEHP in linoleic acid emulsion determined by the thiocyanate method.

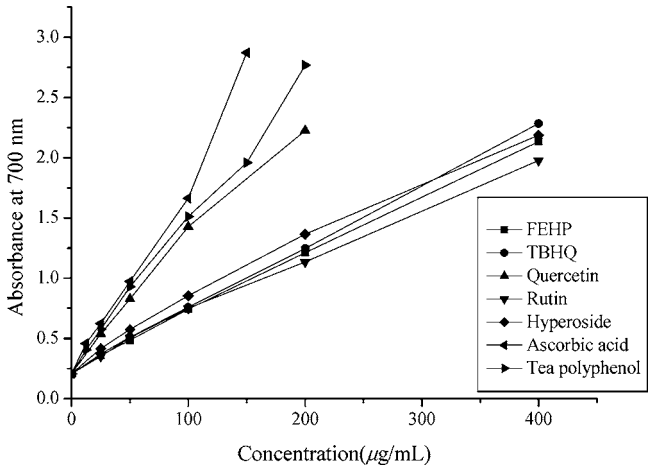


Figure 4. Reducing power of FEHP, quercetin, hyperoside, rutin, ascorbic acid, and tea polyphenol.

dependent. The absorbance of control at 532 nm in this experiment was 0.705 ± 0.096, much lower than that induced by Fenton reaction, which developed an absorbance of 1.847 ± 0.128, and the difference between them was highly statistically significant ($p < 0.01$). The observed results suggested that peroxyl radical was not reactive as hydroxyl radical in inducing peroxidation of liposome, as reported by Wang H. in the

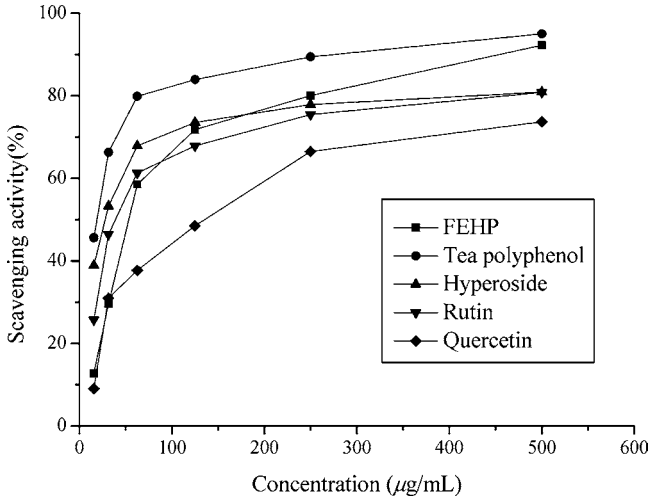


Figure 5. Scavenging effect of different amounts of samples on superoxide anion radical generated in PMS–NADH system.

determination of antioxidant capacity of anthocyanins (*36*), and FEHP was much more effective with Fenton reaction as the source of free radical than with AAPH.

Inhibitory Effect on Deoxyribose Degradation. The inhibitory effect of FEHP on deoxyribose degradation using nonsite-specific and site-specific assay was conducted. As shown in **Figure 7**, a concentration-dependent inhibition against hydroxyl radical induced deoxyribose degradation was observed in both the site-specific and non-site-specific assay, but a greater hydroxyl radical scavenging activity of FEHP was observed in the site-specific assay, the difference between site-specific assay and non-site-specific assay was highly statistically significant ($p < 0.01$). Compared with mannitol, a special hydroxyl radical scavenger, the difference of hydroxyl radical scavenging activity between them was highly statistically significant ($p < 0.01$) In the site-specific assay, at a concentration of 5 mg/mL, FEHP exhibited 92.3% of hydroxyl radical scavenging activity, whereas mannitol only exhibited 20.3% scavenging activity. There was no difference between FEHP and mannitol in the non-site-specific assay ($p > 0.05$).

When hydroxyl radical generated by the Fenton reaction attacks deoxyribose, deoxyribose degrades into fragments that react with TBA on heating at low pH to form a pink color. In nonsite-specific assay, Fe^{3+} and EDTA in the presence of ascorbic acid form an Fe^{2+}-EDTA complex, which is very effective in generating hydroxyl radical in free solution. However, in site-specific assay, when iron is added to the

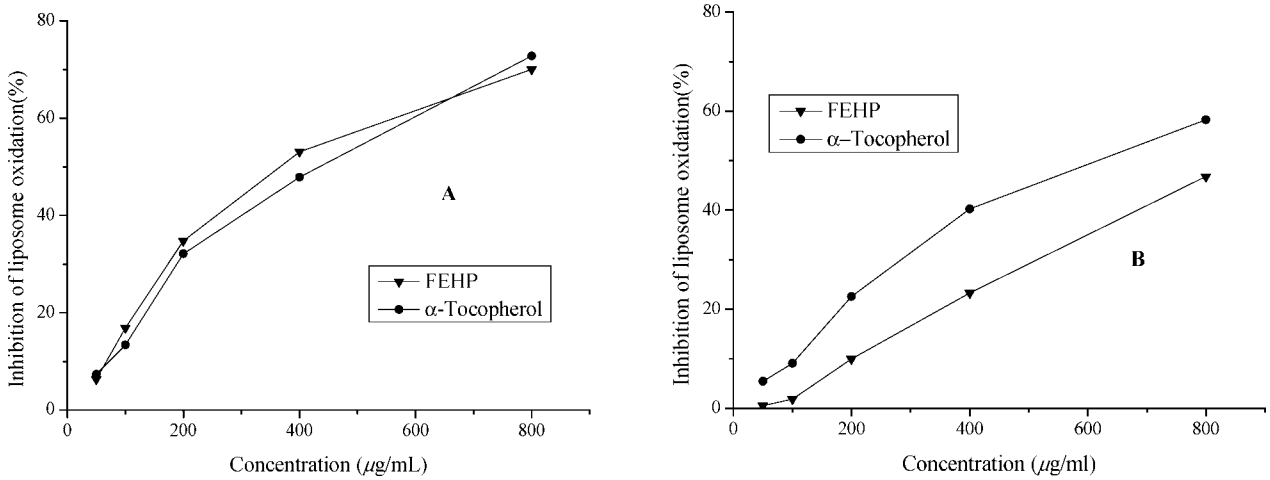


Figure 6. Inhibitory effects of FEHP and α–tocopherol on TBARS formation on the lipid peroxidation of liposome induced by Fe^{3+}/H_2O_2/ascorbic acid (**A**) and AAPH (**B**).

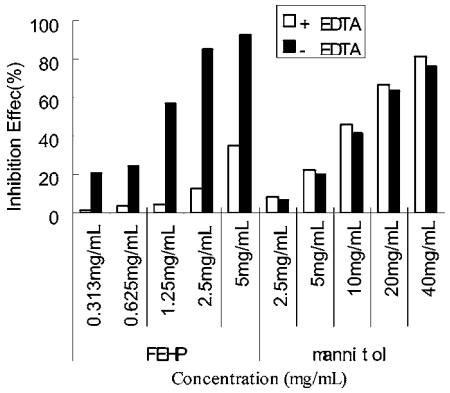


Figure 7. Inhibitory effects of FEHP and mannitol on deoxyribose degradation in site-specific assay (– EDTA) and nonsite-specific assay (+ EDTA).

mixture as ferric chloride instead of ferric-EDTA, some of the Fe^{3+} bind directly into the sugar and hydroxyl radical formed attack sugar immediately. In this assay, iron ions are equally available to both the deoxyribose and the compounds under test. Only compounds that are able to interfere with the ion-binding capacity of sugar and withdraw the iron ions and render them inactive or poorly active in the Fenton reaction can bind iron ions strongly enough to inhibit deoxyribose degradation in the absence of EDTA (*37*). At the same concentration, the inhibition of deoxyribose degradation in the site-specific was greater, which implied that FEHP was a strong metal chelator and a moderate scavenger of hydroxyl radical.

In summary, a flavonoid-rich extract of *Hypericum perforatum* L. was prepared by adsorption on macroporous resin and desorption by ethanol in this study. The primary component was quercetin and its derivatives analyzed by HPLC. In a series of in vitro tests, FEHP exhibited strong antioxidant activity. It acted as a hydrogen-donating agent in the DPPH assay and an electron-donating agent in the iron (III) to iron (II) reducing assay. It prevented the peroxidation of lipid membranes in liposome and inhibited linoleic acid peroxidation. FEHP was also an effective superoxide anion radical scavenger. FEHP inhibited deoxyribose degradation mainly via the chelating iron ions rather than scavenging hydroxyl radical directly. The results of the present study, which demonstrated the radical scavenging activity and antioxidant activity of FEHP, indicated that FEHP might be proposed as a dietary supplement or drug for the treatment of various coronary heart diseases. A detailed work is being undertaken to investigate its antioxidant activity in vivo.

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