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Phenolic content and antioxidant activity of *Phymatopteris hastata*

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Abstract: Various solvent extracts of *Phymatopteris hastata*, a traditional Chinese medicinal material, were screened for their antioxidant activities. Four systems of *in vitro* testing were employed to investigate the antiradical and antioxidant effect, *i.e.*, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) systems, the hydroxyl radical scavenging assay and the reducing power. In addition, butylated hydroxytoluene (BHT), a widely used synthetic antioxidant, was also studied for comparison. The results revealed that the ethyl acetate extract exhibited outstanding antioxidant activities, which was close or even superior to BHT. Furthermore, the total phenolic (TP) and total flavonoid (TF) contents of different extracts were measured, expressed as gallic acid and rutin equivalent, respectively. The antioxidant activities and the TP/TF content of different extracts followed the same order: ethyl acetate extract > butyl alcohol extract > petroleum ether extract, showing a good correlation between the antioxidant activities and the TP/TF content. The results showed that these extracts, especially the ethyl acetate extract, were rich in phenolics and flavonoids and could be considered as natural antioxidants.

Keywords: total phenolic content; total flavonoid content; DPPH; ABTS; hydroxyl radical; reducing power; *Phymatopteris hastata*.

INTRODUCTION

Antioxidants are substances that delay the oxidation process, inhibiting free radical initiated chain polymerization and other subsequent oxidizing reactions,¹ and are widely used in the food, cosmetic and pharmaceutical industries.² Antioxidants play an important role in biological systems because they defend against oxidative damage and participate in the major signaling pathways of cells.³ Of particular importance, they can prevent damage in cells caused by the action of reactive oxygen species.^{4–6}

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Reactive oxygen species (ROS), the major free radicals generated in normal metabolic processes, exist in the form of hydrogen peroxide (H_2O_2), the superoxide anion ($\text{O}_2^{\bullet-}$), the hydroxyl radical ($\bullet\text{OH}$), *etc.*⁷ There is a balance between ROS generation and elimination by endogenous superoxide dismutase, glutathione peroxidase and catalase. However, over-production of ROS, induced by exposure to external oxidant substances or a failure in the defense mechanisms, will cause a variety of biochemical and physiological lesions and often result in metabolic impairment and cell death.⁸ Moreover, impairment to cell structures, DNA, lipids and proteins induced by ROS are associated with more than 30 different disease processes,⁹ including highly disabling vascular pathologies such as cardiovascular disease (CVD)^{10,11} and cardiac failure, alcohol-induced liver disease (ALD) and ulcerative colitis, cancer, and neurodegenerative conditions such as Alzheimer's disease (AD), mild cognitive impairment (MCI) and Parkinson's disease (PD).¹²

Fortunately, the action of active oxygen and free radicals is opposed by a balanced system of antioxidant defenses, hence the presence of antioxidants is essential for human health.⁷ The most commonly used synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) were restricted due to their instability and possible carcinogenicity.^{13–15} Hence, there is growing attention for the search for natural antioxidants, which avoid the side effects and can be used to reduce cellular oxidative lesion in foods and even the human body.^{16–20} Plants, including herbs and spices, have been considered as potential sources for natural antioxidants,^{21,22} because they are rich in phenolic components^{23,24} that have antimutagenic, anticarcinogenic, anti-inflammation, and antioxidant activities.^{25,26}

Phymatopteris hastata (Thunberg) Pichi Sermolli Webbia, which is widespread in south China, is one of the most important traditional medicines belonging to the Polypodiaceae family. It is used as a remedy for a wide range of diseases, for instance, diarrhea, cough, bronchitis, headache, influenza, fever and urinary tract infection. Moreover, it is especially useful in conditions such as carbuncle, furunculosis and viper bites. However, there is no reported information on the antioxidant activity of *P. hastata*. Thus, in the present study, the antioxidant activities of various solvent extracts from *P. hastata* were investigated by employing four *in vitro* test systems: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), hydroxyl radical scavenging assay and reducing power. Furthermore, the total phenolic (TP) and total flavonoid (TF) contents of different extracts were measured to expose the relationship between the TP/TF content and antioxidant activity.

EXPERIMENTAL

Sample and reagents

P. hastata whole plant was collected during the summer of 2009 in the Guangxi Province, China. A voucher specimen was identified by Dr. Songji Wei at the Department of Zhuang Pharmacy, Guangxi Traditional Chinese Medical University. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich Co., St. Louis, MO, USA; 2,2-diphenyl-1-picrylhydrazyl (DPPH) (purity 98 %) was purchased from Wako Chemicals, Japan; rutin and gallic acid standards were purchased from J & K Scientific Ltd., Beijing, China. Other chemicals were purchased from the China National Medicine Group Shanghai Corporation, Shanghai, China. All chemicals and solvents used were of analytical grade.

Preparation of the petroleum ether, ethyl acetate and butyl alcohol extracts

The material was initially air-dried and then reduced to small particles. The particles that passed through a 40-mesh screen were selected for analyses. Fifty grams of air-dried plant material was immersed in 500 mL of ethanol (95 %) and constantly stirred. After 48 h, the filtrate was collected. The extraction was repeated two times more. The combined extracts were then concentrated under reduced pressure at 40 °C using a vacuum rotary evaporator. The above ethanol extract was partitioned with petroleum ether, ethyl acetate and butyl alcohol, and the obtained petroleum ether fractions (PE), ethyl acetate fractions (EE) and butyl alcohol fractions (BE) were collected and concentrated using a vacuum rotary evaporator at 40 °C. The preparation of the petroleum ether, ethyl acetate and butyl alcohol extracts was performed in triplicate.

Determination of the TP and TF contents by spectrophotometric procedures

Determination of the TP content. The total content of soluble phenolics (TP) was determined using the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton,²⁷ with gallic acid as the equivalent. Briefly, the extract solution (0.5 mL, 1 mg mL⁻¹) was diluted with distilled water (46 mL) in a volumetric flask. Folin-Ciocalteu reagent (1 mL) was added and mixed thoroughly. After 3 min, sodium carbonate solution (3 mL, 2 %) was added, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The total phenolic content was determined by comparison with a standard calibration curve for gallic acid, and the results are presented as milligrams of gallic acid equivalent per 1 g (dry weight) of extract (mg GAE g⁻¹ dw). All tests were conducted in triplicate.

Determination of the TF content. The total flavonoid (TF) content was determined following the procedure of Dewanto *et al.*²⁸ with rutin as the equivalent. The extract solution (3 mL, 0.8 mg mL⁻¹) was placed in a 10-mL volumetric flask and then 5 mL of distilled water was added followed by NaNO₂ solution (0.3 mL, 5 %). After 5 min, AlCl₃ solution (0.6 mL, 10 %) was added. After another 5 min, NaOH solution (2 mL, 1 M) was added and volume was made up to the mark with 95 % ethanol. The solution was mixed thoroughly and the absorbance measured at 510 nm. The total flavonoid content was expressed as milligrams of rutin equivalent per 1 g (dry weight) of extract (mg RE g⁻¹ dw). All tests were performed in triplicate.

Determination of the TP and TF content by HPLC analysis

Preparation of standard and sample solutions. The phenolics and flavonoid compounds in stems extracts of *P. hastata* were determined by HPLC, using a Waters 600 diode array

detector (DAD) system equipped with a dualistic pump. The analyses were performed on a Diamonsil C₁₈ column (4.6 mm×250 mm, 2.5 μm). Standard stock solutions of gallic acid and rutin were prepared in 95 % EtOH, at concentration of 0.5 mg mL⁻¹. All sample solutions of PE, EAE and BE (1 mg mL⁻¹) were filtered through a 0.45 μm membrane filter (F type, Millipore), and injected directly.

HPLC analysis of the phenolic compounds. The mobile phase consisted of solvent A (0.3 % acetic acid) and solvent B (acetonitrile). The gradient program was as follow: (0–5 min, 20 % B; 5–10 min, 90 % B; 10–15 min, 10 % B; 15–25 min, 20 % B); the flow rate was 1 mL min⁻¹; the volume injected 20 μL; the temperature 25 °C; UV detection wavelength 280 nm. All experimental data are expressed as mean ±SD values (*n* = 3).

HPLC analysis of the flavonoid compounds. The mobile phase consisted of the solvents of MeOH–water–acetic acid (40:57.5:2.5) at a flow rate of 1.0 mL min⁻¹. The column temperature was set at 25 °C. The injection volume was 20 μL. The UV detection wavelength was 254 nm. All experimental data are expressed as means ±SD values (*n* = 3).

DPPH radical scavenging assay

The scavenging effect of the different fractions on the DPPH radical was measured using a modified version of the method described by Shimada *et al.*²⁹ In brief, the extract solution (20 μL) in 95 % ethanol at different concentrations (0.2, 0.5, 0.8 and 1.2 mg mL⁻¹) was added to 8 mL of a 0.004 % (w/v) solution of DPPH in 95 % ethanol. The scavenging activities on DPPH radical were determined by measuring the absorbance at 515 nm every 10 min until the reaction reached the steady state. The DPPH radical scavenging activity (*S*%) was calculated using the following formula: $S\% = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$, where *A*_{control} is the absorbance of the blank (containing all reagents except the extract solution) and *A*_{sample} is the absorbance of the test sample. The DPPH radical scavenging activity of BHT was also assayed for comparison. All tests were performed in triplicate.

ABTS radical scavenging assay

The antioxidant activities of various solvent extracts in the reaction with the stable ABTS⁺ radical cation were determined according to the method of Delgado-Andrade *et al.*³⁰ Briefly, ABTS⁺ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12–16 h before use. The ABTS⁺ solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 730 nm of 0.70±0.02. After addition of 10 μL of sample to 4 mL of diluted ABTS⁺ solution, the absorbance was read after 30 min. As a positive control, the synthetic antioxidant BHT was used. All the samples were analyzed in triplicate. The ABTS⁺ radical-scavenging activity of the samples was expressed as $S\% = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$, where *A*_{control} is the absorbance of the blank control (ABTS⁺ solution without test sample) and *A*_{sample} is the absorbance of the test sample.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity was performed according to a modified method of Li *et al.*⁵ The extract solution (2 mL, 0.2, 0.5, 0.8 and 1.2 mg mL⁻¹), *o*-phenanthroline solution (1.0 mL, 7.5 mmol L⁻¹), phosphate buffer (5 mL, 0.2 M, pH 6.6), ferrous sulfate solution (1.0 mL, 7.5 mmol L⁻¹) and H₂O₂ (1.0 mL, 0.1 %) were mixed and diluted to 25 mL with distilled water. After incubation at room temperature for 30 min, the absorbance was measured at 510 nm. BHT was used as a positive control. The scavenging percentage (*P*%) was calculated as $P\% = ((A - A_1) / (A_2 - A_1)) \times 100$, where *A*, *A*₁ and *A*₂ are the absorbance value of the system with all solutions including H₂O₂ and extract solution, the system without extract

solution, and the system without H₂O₂ and extract solution, respectively. All tests were performed in triplicate.

Measurement of the reducing power

The reducing power of *P. hastata* was determined according to the method of Gulcin.⁴ Fractions solutions (100 µL) in 95 % ethanol (0.2, 0.5, 0.8 and 1.2 mg mL⁻¹) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 7.4) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1 %). After incubation at 50 °C for 20 min, 2.5 mL of trichloroacetic acid (10 %, w/v) was added and the mixture centrifuged at 3000 rpm for 10 min. 2.5 mL of the upper layer of the above solution was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1 %), and then the absorbance was measured at 700 nm. The higher is the absorbance value, the stronger is the reducing power. The reducing power of BHT was also assayed for comparison. All determinations were performed in triplicate.

Statistical analysis

All tests were conducted in triplicate. The results were expressed as means ±SD. Analysis of the variance and significant differences among the means were tested by one-way ANOVA, using SPSS (Version 13.0 for Windows, SPSS Inc., Chicago, IL). *P* values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

TP and TF contents determined by spectrophotometric procedures

The total phenolics content (TP) and the total flavonoid content (TF) of extracts were determined from regression equations for the calibration curves ($y = 0.0796x + 0.0091$, $R^2 = 0.9925$) and ($y = 0.8836x - 0.0255$, $R^2 = 0.9998$), respectively. The TP and TF content of the PE, EE, and BE are listed in Table I. The TP content, as determined by the Folin-Ciocalteu method, is reported as gallic acid equivalents, while the TF content is expressed as rutin equivalents. It is well known that phenolic and flavonoid compounds are potential antioxidants and free-radical scavengers; hence there should be a close correlation between the TP/TF content and the antioxidant activity. The TP/TF content of the three extracts was in the following order: EE > BE > PE.

TABLE I. TP content and TF content of different extracts from *P. hastata* determined by spectrophotometric procedures (results are the mean ± standard deviation of three parallel measurements)

Sample	TP / mg GAE g ⁻¹ dw	TF / mg RE g ⁻¹ dw
EE	70.2±0.28	180.6±0.40
BE	53.6±0.16	98.0±0.22
PE	49.4±0.20	51.2±0.19

TP and TF contents determined by HPLC analysis

Reverse phase-HPLC coupled with UV-Vis DAD was employed to separate, identify and quantify the phenolic compounds in the fractions of *P. hastata*. The concentrations were determined by calculating the HPLC peak areas, which are

proportional to the amount of analyte in a peak, and are presented as the mean of three determinations, which were highly repeatable. The standard curves for gallic acid and rutin were $y = 3397.9 + 10474.7x$ ($R^2 = 0.990$) and $y = 4.89 + 16640.2x$ ($R^2 = 0.998$), respectively. The chromatogram of the reference standards gallic acid and rutin is shown in Fig. 1. These compounds were identified in the PE, EE, and BE according to their retention times and the spectral characteristics of their peaks compared to those of the standards. As shown in Table II, the extract with the highest TP amount was EE (83.01 ± 0.56 mg GAE g^{-1} dw), followed by BE (62.92 ± 0.52 mg GAE g^{-1} dw) and PE (36.83 ± 0.78 mg GAE g^{-1} dw). The same trend was observed for the TF content. Of these three extracts, EE contained the higher TP content (83.01 ± 0.56 mg GAE g^{-1} dw) and TF content (73.11 ± 0.61 mg RE g^{-1} dw) than BE and PE, indicating that EE might have the most outstanding antioxidant activity of the various solvent extracts.

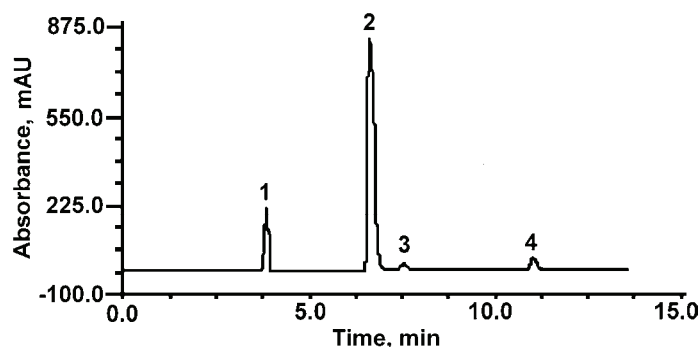


Fig. 1. HPLC chromatogram of the reference standards; 1) gallic acid, 2) rutin and 3) and 4) unknown residues.

TABLE II. TP content and TF content of different extracts from *P. hastata* determined by HPLC (results are the mean \pm standard deviation of three parallel measurements; ND – not detected)

Sample	TP / mg GAE g^{-1} dw	TF / mg RE g^{-1} dw
EE	83.01 ± 0.56	73.11 ± 0.61
BE	62.92 ± 0.52	55.79 ± 0.46
PE	36.83 ± 0.78	ND

DPPH radical scavenging activity

DPPH, a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by an antioxidant, has been widely employed to investigate the free-radical scavenging ability of various antioxidants because of its advantages of ease, economy and sensitivity to detect active compounds at low concentrations.^{21,31} The DPPH radical scavenging activity of the three extracts is shown in Fig. 2, compared with the synthetic antioxidant BHT. As can be seen,

the scavenging effect of EE, BE, PE and BHT increased with increasing concentration. For EE, a sharp increase of its DPPH scavenging activity (43.2–84.2 %) was observed, as its concentration increased from 0.2 to 1.2 mg mL⁻¹. The *IC*₅₀ values on the DPPH radical of BHT and the extracts of *P. hastata* are given in Table III. EE, PE and BE possessed significant scavenging activity on the DPPH radical. Moreover, EE exhibited the highest DPPH scavenging effect (*IC*₅₀ = 0.25 mg mL⁻¹), which is superior to that of BHT (*IC*₅₀ = 0.45 mg mL⁻¹), indicating that the EE was a prominent scavenger of the DPPH radical.

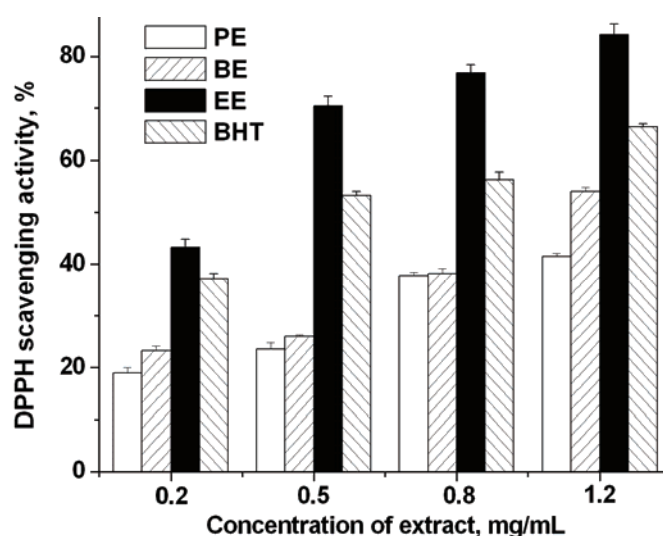


Fig. 2. DPPH radical scavenging activity of the various solvent extracts from *P. hastata*. The results are the mean \pm SD of three parallel measurements. The values are significantly different ($P < 0.05$).

TABLE III. *IC*₅₀ values of different extracts from *P. hastata* and BHT

Extract	<i>IC</i> ₅₀ / mg mL ⁻¹		
	DPPH	ABTS ⁺	*OH
BHT	0.45	0.38	0.59
EE	0.25	0.38	0.62
BE	1.35	0.54	1.64
PE	2.09	33.70	1.66

ABTS⁺ radical scavenging activity

This method is based on the inhibition by antioxidants of the absorbance of the radical cation of ABTS, which has a characteristic long-wavelength absorption spectrum showing a maximum at 734 nm. The experiments, which can be used in both organic and aqueous solvent systems, are realized using a decolorization assay, which involves the generation of the ABTS⁺ chromophore by the

oxidation of ABTS⁺ with potassium persulfate.¹² The ABTS⁺ scavenging capacities of EE, BE, PE and BHT were measured and are compared in Fig. 3, and their IC_{50} values on ABTS⁺ are presented in Table III.

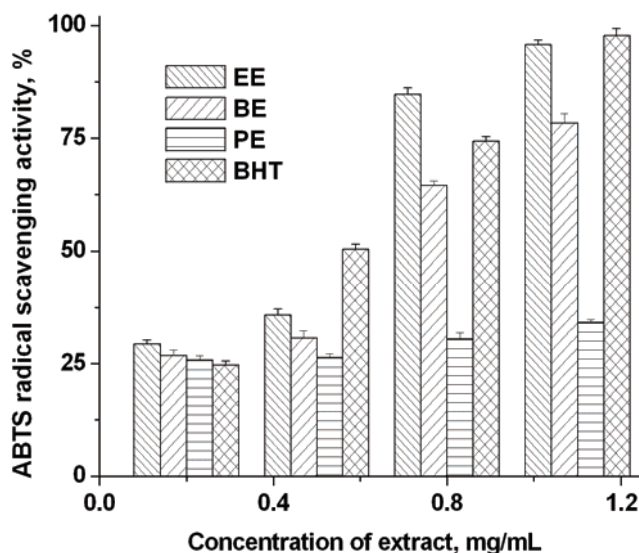


Fig. 3. ABTS⁺ scavenging activity of the various solvent extracts from *P. hastata*. The results are the mean \pm SD of three parallel measurements. The values are significantly different ($P < 0.05$)

The ABTS⁺ scavenging activities of all extracts and BHT increased in a concentration dependent manner. The IC_{50} values decreased in the following order: PE (33.70 mg mL⁻¹) < BE (0.54 mg mL⁻¹) < EE/BHT (0.38 mg mL⁻¹). Of the three extracts, EE exhibited the most effective scavenging ability, which was the same as that of BHT, while the lowest one was found to be the PE. The order of scavenging activities on the ABTS radical of the three extracts was similar to those on DPPH. The differences of the ABTS⁺ scavenging activities of EE, BE and PE indicated that the extraction media significantly influenced the antioxidant abilities of the extracts.

Hydroxyl radical scavenging activity

Hydroxyl radicals, which could be formed from the superoxide anion and hydrogen peroxide, are the most reactive and predominant radicals generated endogenously during aerobic metabolism among the reactive oxygen species (ROS). The Fenton reaction is the most important mechanism for hydroxyl radical formation *in vitro*, in which a transition metal is involved as a pro-oxidant in the catalyzed decomposition of superoxide and hydrogen peroxide.⁷

In present study, the scavenging activity on hydroxyl radicals of EE, BE and PE was investigated (Fig. 4), using ferrous sulfate as the pro-oxidant. BHT was used as a positive control. The IC_{50} values of BHT and extracts of *P. hastata* on hydroxyl radicals are presented in Table III. The three extracts and BHT exhibited strong concentration-dependent scavenging activities on the hydroxyl radical. In the extracts from *P. hastata*, EE was found to be the most powerful scavenger of hydroxyl radicals, with an IC_{50} value of 0.62 mg mL^{-1} , which was similar to that of BHT ($IC_{50} = 0.59 \text{ mg mL}^{-1}$). It is worth mentioning that EE showed an inhibition of 10.4 % at a concentration as low as 0.2 mg mL^{-1} . The scavenging activities of the three extracts were in the order: $EE > BE > PE$.

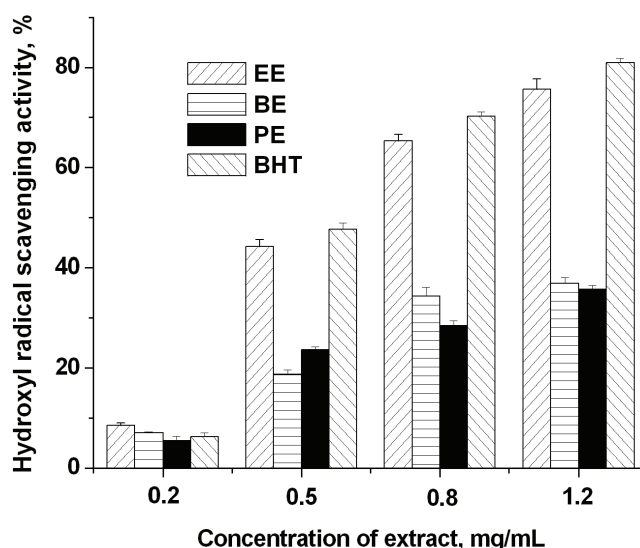


Fig. 4. Hydroxyl radical scavenging activity of the various solvent extracts from *P. hastata*. The results are the mean \pm SD of three parallel measurements. The values are significantly different ($P < 0.05$).

Reducing power

The reducing capacity of a compound, which depends on the presence of reductants that exhibit antioxidant potential by breaking the radical chain and donating a hydrogen atom, may serve as a significant indicator of its potential antioxidant activity.³² The reducing capability of the extract samples was monitored by measuring the formation of Perls' Prussian blue, absorption at 700 nm, due to the transformation of Fe^{3+} /ferricyanide complex to Fe^{2+} /ferrous form in the presence of reductants in the extracts. Increased absorbance of the reaction mixture indicates increased reducing power.³³

The reducing power of various solvent extracts from *P. hastata* is shown in Fig. 5. EE, BE and PE exhibited different degrees of electron donating capacities

in a concentration-dependent manner, whereby EE was the most outstanding at various concentrations. The reducing capacities at 700 nm for EE, BE, PE and BHT were 0.98, 0.63, 0.52 and 1.02, respectively. Therefore, the reducing power order was: BHT > EE > BE > PE. The absorbance of EE was similar to that of BHT, indicating that EE possessed a comparative reducing power compared to BHT. The trend in the reducing power of the various solvent extracts from *P. hastata* was similar to those of their DPPH, ABTS, hydroxyl radical scavenging activities and the content of TP/TF, indicating that there is a correlation between the TP/TF content and the antioxidant activities of the various solvent extracts from *P. hastata*.

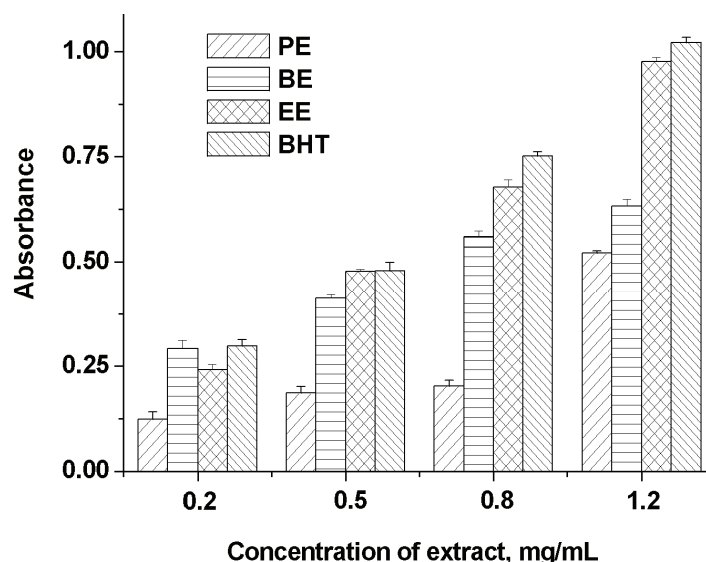


Fig. 5. Reducing power of the various solvent extracts from *P. hastata*. The results are the mean \pm SD of three parallel measurements. The values are significantly different ($P < 0.05$).

CONCLUSIONS

In the present investigation, extracts of *P. hastata* exhibited outstanding scavenging effects on DPPH, ABTS and hydroxyl radical, and possessed a strong reducing power. EE, which had the highest total phenolic (TP) and total flavonoid (TF) contents, was shown to be the most efficient extract and was superior to butylated hydroxytoluene. The TP/TF content of the different extracts and their antioxidant activities in all the tested systems followed the same order: EE > BE > PE, showing there was a significant correlation between antioxidant activities and TP/TF content. The results indicated that EE, BE and PE possessed sufficient phenolic compounds and exhibited excellent antioxidant activities. Based on the above results, various solvent extracts of *P. hastata*, especially EE extract,

were found to be excellent scavengers of free radical and possess remarkable antioxidant ability. The results showed that these extracts could be a promising source of natural antioxidants in the food industry and may be useful for reducing the risks of chronic diseases and preventing diseases progression.

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ИЗВОД

ФЕНОЛНИ САДРЖАЈ И АНТИОКСИДАТИВНА АКТИВНОСТ БИЉКЕ
*Phymatopteris hastata*WEI SU¹, PEIYUAN LI², LINI HUO², CAIYING WU¹, NANA GUO¹ и LIANGQUAN LIU¹¹College of Chemistry and Life Science, Guangxi Teachers Education University, Nanning u ²College of Pharmacy, Guangxi traditional Chinese Medical University, Nanning, China

Испитивана је антиоксидативна активност екстракта традиционалне лековите кинеске биљке *Phymatopteris hastata*. Четири методе су коришћене за одређивање антирадикалског и антиоксидативног ефекта: са 2,2-дифенил-1-пикрилхидразидом (DPPH), са 2,2'-азинобис(3-етилбензотиазолин)-6-сулфонском киселином (ABTS), тест за хватање слободних хидроксилних радикала и тест за одређивање редукујуће способности. Резултати су поређени са ефектима бутилованог хидрокситолуена (BHT), синтетичког антиоксиданта. Показано је да етилацетатни екстракт има изузетну антиоксидативну активност, сличну или већу него BHT. Укупни садржај фенола (TP) и флавоноида (TF) је измерен и изражен као еквивалент галне киселине, односно рутина. Антиоксидативна активност и TP/TF садржај различитих екстракта су били у следећем низу: етилацетатни > бутилалкохолни > петролетарски екстракт, указујући на добру корелацију између антиоксидативне активности и садржаја TP/TF. Сви екстракти, а посебно етилацетатни, су богати фенолима и флавоноидима и могу се сматрати природним антиоксидантима.

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