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Original Article

Protective affect against hydroxyl radical induced DNA damage and antioxidant mechanism of *Begonia fimbristipula*

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Key Words
Begonia fimbristipula; DNA damage; Hydroxyl radical

Abstract
As a tea or functional vegetable in southeast China, *Begonia fimbristipula* Hance possesses various pharmacological effects including antioxidant ability. However, its antioxidant effect has not been reported yet. The objective of this work was to investigate its antioxidant ability, then further discuss the antioxidant mechanism. *B.fimbristipula* Hance was extracted by methanol to obtain methanol extract of *B.fimbristipula* Hance (EBFH). EBFH was then measured by various antioxidant methods, including DNA damage assay, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) assay, superoxide anion radical ($O_2^{\bullet-}$) scavenging assay, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate radical ($ABTS^{\bullet+}$) assay, Fe^{3+}-reducing assay and Cu^{2+}-reducing assay. The total phenolic content of EBFH was estimated using Folin-Ciocalteu method as well. Our results revealed that EBFH could effectively protect against hydroxyl-induced DNA damage. In addition, it could also scavenge $O_2^{\bullet-}$, DPPH• and $ABTS^{\bullet+}$ radicals, and reduce Fe^{3+} and Cu^{2+}. The total phenols content of EBFH was 133.25 ± 2.88 pyrogallol/g. In conclusion, *B.fimbristipula* Hance can effectively protect against hydroxyl-induced DNA damage. One mechanism of this protective effect may be radical scavenging via donating hydrogen atom (H•), donating electron (e). Its antioxidant ability may be mainly attributed to the total phenols, especially cyanidin-3-O-glucoside.

INTRODUCTION

As the most important biomacromolecule, DNA can be easily damaged by hydroxyl (•OH) and other reactive oxygen species (ROS) then lead to various diseases or disorders [1]. Therefore, it is vital to search for potential therapeutic agents for DNA oxidative damage. Over the last two decades, much attention has been focused on the antioxidant efficacy of medicinal plants, especially Chinese medicinal herbals.

Begonia fimbristipula Hance (Fig.1) is traditionally used as a tea or functional food in southeast China. From the viewpoint of traditional Chinese Medicine, *B.fimbristipula* has various healthcare functions, including heat-clearing, detoxifying and so on [2]. Modern pharmacology indicated protective effects against ionizing radiation damage [3] and diabetic nephropathy [4]. Undoubtedly, these healthcare functions or pharmacological effects are associated with the antioxidant ability. However, the antioxidant ability of *B.fimbristipula* has not been reported so far.

The purpose of the present study was to systematically investigate the antioxidant ability of *B.fimbristipula*, especially the protective effect against DNA damage, then to further discuss the mechanism.

MATERIALS AND METHODS

Plant material

Dried *Begonia fimbristipula* Hance was purchased from Zhaoqing Dinghutang Trading Co (Guangdong, China). A voucher specimen was deposited in our laboratory.

Chemicals

1,1-diphenyl-2-picryl-hydrazl (DPPH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate (ABTS), butylated hydroxyanisole (BHA), (±)-6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), DNA

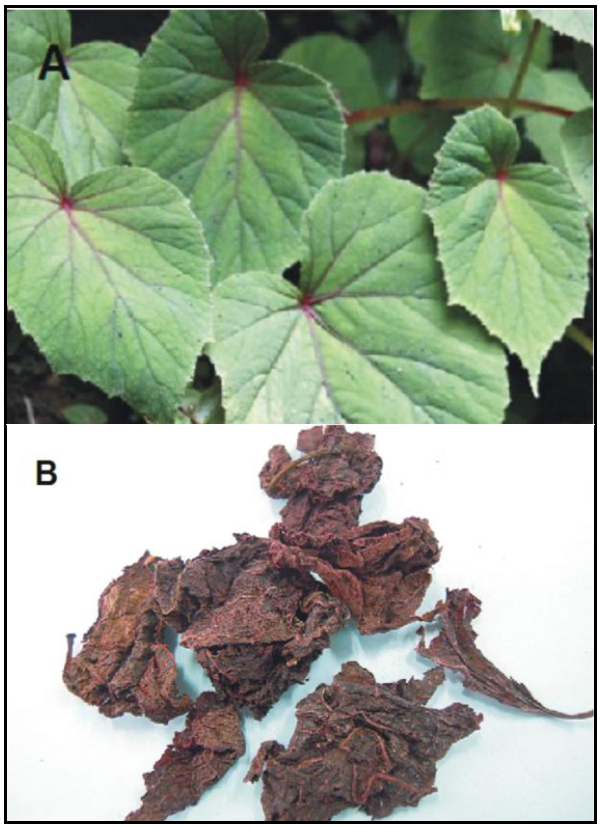

Figure 1. Plant of *Begonia fimbristipula* Hance (A) and its dried herb (B). (Fig.1A was obtained from www.nature-museum.net and Fig.1B was delivered by Lu Han).

sodium salt (fish sperm), 2,9-dimethyl-1,10-phenanthroline (neocuproine) and Folin-Ciocalteu reagent were purchased from Sigma (Sigma-Aldrich Shanghai Trading Co, China). Proanthocyanidin was obtained from Aladdin Chemical Co. (Shanghai, China). Other chemicals used in this study were of analytic grade.

Preparation of extracts from *Begonia fimbristipula*

Begonia fimbristipula Hance was powdered then extracted by methanol using a Soxhlet extractor for 6 h. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was then concentrated to dryness under reduced pressure to yield methanol extract of *Begonia fimbristipula* Hance (EBFH). It was stored at 4°C for analysis.

Protective effect against hydroxyl-induced DNA damage

The experiment was conducted according to our method [5]. Briefly, sample was dissolved in methanol at 4 mg/ml. Various amounts (10-50 µl) of sample methanol solutions were then separately taken into tubes. After evaporating the sample solutions in tubes to dryness, 300 µl of phosphate buffer (0.2 mol/l, pH 7.4) was added to the sample residue. Subsequently, 100 µl Na_2EDTA (0.5 mmol/l), 50 µl $FeCl_3$ (3.2 mmol/l), 75 µl H_2O_2 (33.6 mmol/l) and 50 µl DNA sodium (10 mg/ml) were added. The reaction was initiated by adding 50 µl ascorbic acid (12 mmol/l). After incubation in a water bath at 50°C for 20 min, the reaction was terminated by adding 250 µl trichloro-acetic acid (10 g/100 ml in water). The color was then developed by addition of 150 µl of 2-thiobarbituric acid (TBA) (0.4 mol/l in 1.25% NaOH aqueous solution) and heating in an oven at 105°C for 15 min. The mixture was cooled and absorbance was measured at 530 nm against the buffer (as blank). The percent of protection against DNA damage is expressed as follows:

$$\textbf{Protective effect (\%)} = (A_0 - A) / A_0 \times 100$$

-$\mathbf{A_0}$**;** the absorbance of the mixture without sample

-**A;** the absorbance of the mixture with sample

Superoxide anion radical-scavenging assay

Measurement of $O_2\bullet^-$ scavenging activity was based on our method [6]. Briefly, the sample was dissolved in ethanol at 1 mg/ml. The sample solution (*x* µl, where *x* is 0, 50, 100, 150, 200, 250, 300 and 350) was mixed with '2950 – *x*' µl Tris-HCl buffer (0.05 mol/l, pH 7.4) containing Na_2EDTA (1 mmol/l). 50 µl pyrogallol (60 mmol/l in 1 mmol/l HCl) was added and the mixture was shaken at room temperature immediately. The absorbance at 325 nm of the mixture was measured (Unico 2100 UV Spectrophotometer, Shanghai, China) against the Tris-HCl buffer as blank every 30 seconds for 5 min. The $O_2\bullet^-$ scavenging ability was calculated as:

$$\textbf{Inhibition (\%)} = \frac{(\Delta A_{325nm,control}/T) - (\Delta A_{325nm,sample}/T)}{(\Delta A_{325nm,control}/T)} \times 100$$

-$\mathbf{\Delta A_{325nm,control}}$**;** increase in ΔA_{325nm} of the mixture without sample

-$\mathbf{\Delta A_{325nm,sample}}$**;** increase in ΔA_{325nm} of the mixture with sample

-**T** = 5 min

The experiment temperature was 37°C. Caffeic acid and Trolox were used as positive controls.

DPPH• scavenging assay

DPPH• scavenging activity was determined as previously described by Li [7]. Briefly, 1 ml DPPH methanol solution (0.1 mM) was mixed with 0.5 ml sample methanol solution (6.67-46.67 µg/ml). The mixture was kept at room temperature for 30 min, and then measured with a spectrophotometer (Unico 2100) at 519 nm. The DPPH• inhibition percentage was calculated as:

$$\textbf{Inhibition (\%)} = (A_0 - A) / A_0 \times 100$$

-$\mathbf{A_0}$**;** the absorbance without sample

-**A;** the absorbance with sample

Caffeic acid and BHA were used as the positive controls.

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ABTS•$^+$ scavenging assay

The ABTS•$^+$ scavenging activity was measured as described previously [8] with some modifications. ABTS•$^+$ was produced by mixing 0.2 ml ABTS diammonium salt (7.4 mmol/L) with 0.2 ml potassium persulfate (2.6 mmol/l). The mixture was kept in the dark at room temperature for 12 h to allow completion of radical generation, then diluted with 95% ethanol (about 1:50) so that its absorbance at 734 nm was 0.7 ± 0.02. To determine the scavenging activity, 1.2 ml aliquot of diluted ABTS•$^+$ reagent was mixed with 0.3 ml of sample ethanol solution (6.67-33.33 μg/ml). After incubation for 6 min, the absorbance at 734 nm was read on a spectrophotometer (Unico 2100). The percentage inhibition was calculated as:

$$\textbf{Inhibition (\%)} = (A_0 - A) / A_0 \times 100$$

-A_0; absorbance of the mixture without sample

-A; absorbance of the mixture with sample

Reducing power assays

Ferric (Fe^{3+}) reducing power was determined according to the method of Oyaizu [9]. In brief, sample solution *x* μl (1 mg/ml, *x* = 80, 160, 240, 320 and 400) was mixed with '$1400 - x$' μl Na_2HPO_4/KH_2PO_4 buffer (0.2 mol/l, pH 6.6) and 1 ml $K_3Fe(CN)_6$ aqueous solution (1 g/100 ml). After incubated at 50ºC for 20 min, the mixture was added by 1 ml of trichloroacetic acid (10 g/100 ml), and then centrifuged at 3500 rpm for 10 min. As soon as 1 ml supernatant was aliquoted into 1 ml $FeCl_3$ (0.1 g/100 ml in distilled water) the timer was started. At 90 seconds, absorbance of the mixture was read at 700 nm (Unico 2100). Samples were analyzed in groups of three, and when the analysis of one group has finished, the next group of three samples was aliquoted into $FeCl_3$ to avoid oxidization by air. The relative reducing ability of the sample was calculated by using the formula:

$$\textbf{Relative reducing effect (\%)} = (A - A_{min}) / (A_{max} - A_{min}) \times 100$$

-A_{max}; the maximum absorbance at 700 nm

-A_{min}; the minimum absorbance in the test

-A; the absorbance of sample

The cupric ion (Cu^{2+}) reducing capacity was determined by the previously used method [10] with minor modifications. Briefly, 125 μl $CuSO_4$ aqueous solution (0.01 mol/l), 125 μl neocuproine ethanolic solution (7.5 mmol/l) and '$750 - x$' μl CH_3COONH_4 buffer solution (0.1 mol/l, pH 7.5) were brought to test tubes. Then, different volumes of samples (1 mg/ml, *x* = 10-100 μl) were added to the tubes. Then, the total volume was adjusted to 1000 μl with the buffer and mixed vigorously. Absorbance against a buffer blank was measured at 450 nm after 30 min (Unico 2100). The relative reducing power of the sample as compared with the maximum absorbance was calculated by following formula:

$$\textbf{Relative reducing effect (\%)} = (A - A_{min}) / (A_{max} - A_{min}) \times 100$$

-A_{max}; maximum absorbance at 450 nm

-A_{min}; minimum absorbance in the test

-A; absorbance of sample

Determination of total phenolic content

The total phenolic content of EBFH was determined using the Folin-Ciocalteu method [11] with a little modifications. In brief, 0.5 ml sample ethanol solution (0.2 mg/ml) was mixed with 0.5 ml Folin-Ciocalteu reagent (0.25 mol/l). After incubation for 3 min, 1 ml of Na_2CO_3 aqueous solution (15%, w/v) was added. After standing at room temperature for 30 min, the mixture was centrifuged at 3500 rpm for 3 min. The absorbance of the supernatant was measured at 760 nm (Unico 2100). The determinations were performed in triplicate, and the calculations were based on a calibration curve obtained with pyrogallol. The results were expressed as pyrogallol equivalents in milligrams per gram of extract.

Statistical analysis

Data are given as the mean ± standard deviations of three measurements. The IC_{50} values were calculated by linear regression analysis. All linear regression in this paper was analyzed by Origin 6.0 professional software (OriginLab, Northampton, MA, USA). Significant differences were performed using the t-test ($P < 0.05$). The analysis was performed using SPSS software (v.17, SPSS, Chicago, IL, USA).

RESULTS

Protective effect against hydroxyl-induced DNA damage

Our data revealed that EBFH along with the positive controls increased the percentages of protection in a dose-dependent manner (Fig.2A) and the IC_{50} value of EBFH was 317.23 ± 11.36 μg/ml (Table 1).

Superoxide anion radical-scavenging assay

As shown in Fig.2B, EBFH exhibited a dose-dependent curve in O_2•$^-$ scavenging assay based on pyrogallol autoxidation at pH 7.4. In terms of the dose response curve, its IC_{50} was calculated as 149.03 ± 0.3 μg/ml (Table 1).

DPPH• and ABTS•$^+$ radicals scavenging assay

DPPH and ABTS assays have been widely used to determine the free radical scavenging activity of various antioxidant samples. Both DPPH• and ABTS•$^+$ are stable free radicals which dissolve in methanol or ethanol, however, both of them can be scavenged by an antioxidant. Our data showed that EBFH presented a good dose-dependent manner in both DPPH• and ABTS•$^+$ radical-scavenging assays (Figs.2C&D). The IC_{50} values were calculated as 32.34 ± 0.58 and

9.95 ± 0.47 μg/ml, respectively for DPPH• and ABTS•$^{+}$ (Table 1).

Fe^{3+} and Cu^{2+} reducing power assays

The dose-response curves in Figs.2E&F suggested that EBFH increased Fe^{3+} and Cu^{2+} reducing percentages in a dose-response manner and the IC_{50} values were 125.87 ± 0.28 and 65.65 ± 0.20 μg/ml, respectively for Fe^{3+}- and Cu^{2+}-reducing (Table 1).

Determination of total phenols

The calculation of total phenols was based on a calibration curve obtained with pyrogallol (not shown) and the result was expressed as pyrogallol equivalents in milligrams per gram of extract. According to the regression equation **'*y* = 0.1230*x* + 0.1122'**, the content of total phenols in EBFH was calculated as 133.25 ± 2.88 mg pyrogallol/g.

Table 1. The IC_{50} values of methanol extract from *Begonia fimbristipula* Hance (EBFH) (μg/ml)

	EBFH	Positive controls		
		Caffeic acid	Butylated hydroxyanisole	Proanthocyanidin
Protecting DNA damage	317.23 ± 11.36^{b}	285.23 ± 5.57^{a}	360.39 ± 28.67^{b,*}	475.35 ± 18.07^{c}
O_2•$^{-}$ scavenging	149.03 ± 0.3^{b}	25.26 ± 0.07^{a}	264.25 ± 2.57^{c,*}	117.11 ± 3.62^{d}
DPPH•·scavenging	32.34 ± 0.58^{c}	3.98 ± 0.07^{a}	6.36 ± 0.22^{b}	5.36 ± 0.63^{b}
ABTS•$^{+}$·scavenging	9.95 ± 0.47^{b}	3.35 ± 0^{a}	3.35 ± 0^{a}	1.04 ± 0.04^{c}
Fe^{3+} reducing	125.87 ± 0.28^{c}	27.52 ± 1.07^{b,*}	19.34 ± 0.71^{a}	49.28 ± 3.24^{d}
Cu^{2+} reducing	65.65 ± 0.2^{c}	4.85 ± 0.09^{a}	13.37 ± 0.34^{b}	4.99 ± 0.25^{a}

IC_{50} value is defined as the concentration of 50% effect percentage and expressed as mean ± SD (n = 3). Mean values with different superscripts in the same row are significantly different (P < 0.05) and positive controls of values with superscripts '*' are Trolox.

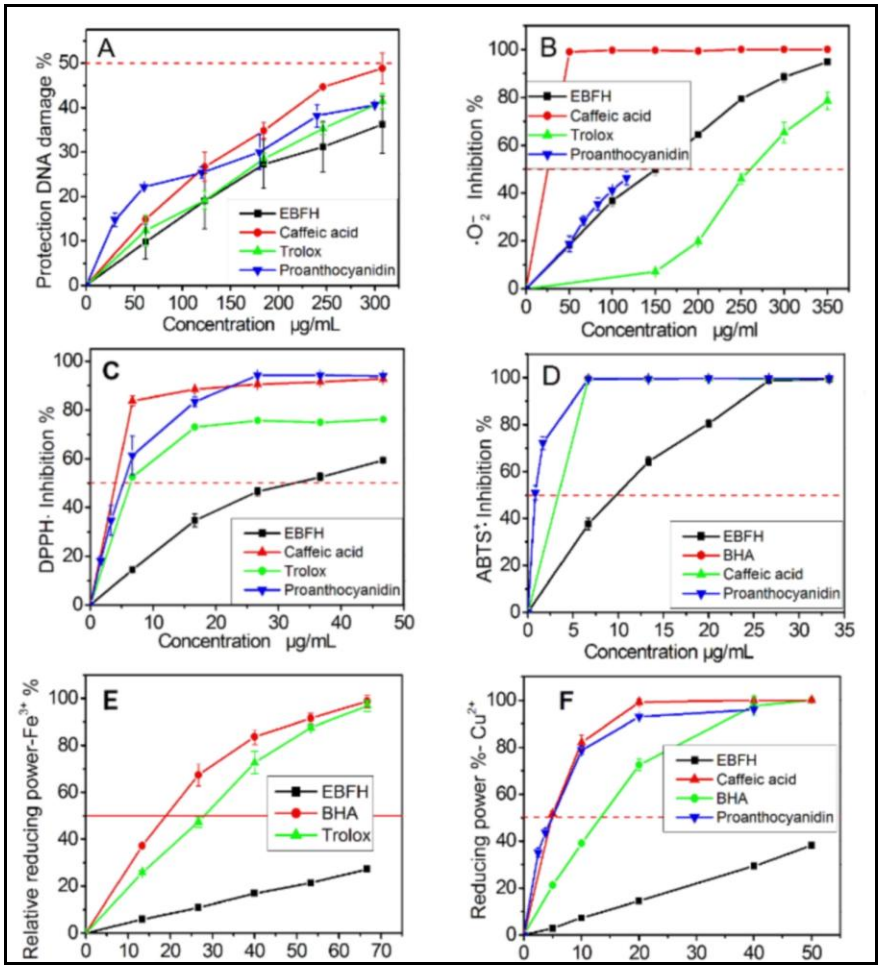

Figure 2.
The dose response curves of EBFH in the antioxidant assays:
(A) protective effect against DNA damage;
(B) superoxide anion (O_2•$^{-}$) scavenging;
(C) DPPH• scavenging;
(D) ABTS•$^{+}$ scavenging;
(E) Fe^{3+} reducing;
(F) Cu^{2+} reducing.
EBFH, absolute methanol extract of *Begonia fimbristipula* Hance;
BHA, butylated hydroxyanisole.
Trolox, caffeic acid, BHA and proanthocyanidin were used as the positive controls.
Each value is expressed as mean ± SD (n = 3).

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DISCUSSION

It is well known that hydroxyl radical (•OH) is generated in human body via Fenton reaction (Equation 1).

$$Fe^{2+} + H_2O_2 \rightarrow \bullet OH + OH^- + Fe^{3+} \quad \text{(Equation 1)}$$

Since •OH has extreme reactivity, it can easily damage DNA to give rise to malondialdehyde (MDA) and various oxidative lesions [12, 13]. If MDA combines TBA, it will produce thiobarbituric acid reactive substances (TBARS) which present a maximum absorbance at 530 nm [14].

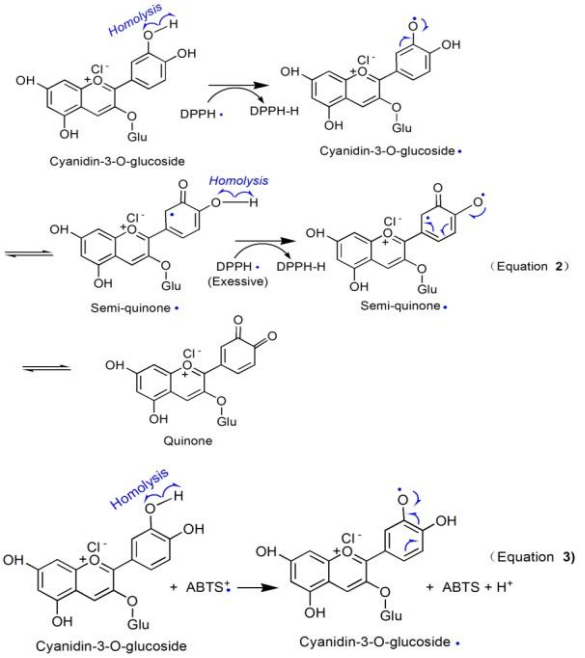
On the other hand, as the oxidative lesions mentioned above have no conjugative system in the molecules, they cannot be detected by a spectrophotometer at 530 nm. It means that these oxidative lesions can bring about no interference with the determination of MDA. Hence, the value of A_{530nm} can evaluate the amount of MDA, and ultimately reflect the extent of DNA damage. Based on the formula **"Protective effect = $(1 - A/A_0) \times 100\%$"** it can be deduced that the decrease of A_{530nm} value indicates a protective effect against DNA damage.

Previous works have shown that there are two approaches for natural antioxidants to protect DNA oxidative damage: one is to scavenge the •OH, then to reduce its attack; the other is to fast repair the deoxynucleotide radical cations which were damaged by •OH [15]. To further confirm whether the protective effect of EBFH was associated with its radical scavenging ability, we determined the $O_2\bullet^-$, DPPH• and $ABTS\bullet^+$ scavenging abilities.

Like •OH, $O_2\bullet^-$ occur in human body as well. In the study, however, EBFH could also effectively scavenge $O_2\bullet^-$. Therefore, direct radical scavenging may be one of approaches for EBFH to protect DNA oxidative damage.

To explore the mechanism for EBFH to scavenge radicals, DPPH• and $ABTS\bullet^+$ scavenging abilities of EBFH were further measured. The previous study suggested that DPPH• may be scavenged by an antioxidant through donation of hydrogen atom (H•) to form a stable DPPH-H molecule [16]. For example, cyanidin-3-O-glucoside which has been isolated from EBFH [17] may scavenge DPPH• via the mechanism shown in Equation 2 [18, 19].

Similarly, EBFH could also scavenge $ABTS\bullet^+$ effectively. However, $ABTS\bullet^+$ scavenging is regarded as an electron (e) transfer reaction [20]. Therefore, the possible mechanism for cyanidin-3-O-glucoside to scavenge $ABTS\bullet^+$ may be via the proposed reaction in Equation 3 to yield cyanidin-3-O-glucoside radical which can also further converted to semi-quinone or quinone under excessive exposure to $ABTS\bullet^+$.



Taken together, the fact that EBFH could effectively scavenge both DPPH• and $ABTS\bullet^+$ suggests that EBFH exerted its radical-scavenging action by donating hydrogen atom (H•) and electron (e).

Finally, we determined the Fe^{3+} and Cu^{2+} reducing powers of EBFH. The data which suggests an effective reducing power of EBFH obviously agrees with the radical-scavenging assays mentioned above. Previous studies have shown that total phenols can be responsible for the antioxidant ability in plants. We then determined the content of total phenols in EBFH and our results indicated a high amount of total phenols. In fact, at least 5 phenolic compounds (Fig.3) have been isolated from *Begonia fimbristipula* Hance until now, including cyanidin chloride, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, rutin and catechin [17]. Among them, cyanidin-3-O-glucoside presents the highest amount [21] and it is regarded as the main bioactive antioxidant compound of *B.fimbristipula* Hance.

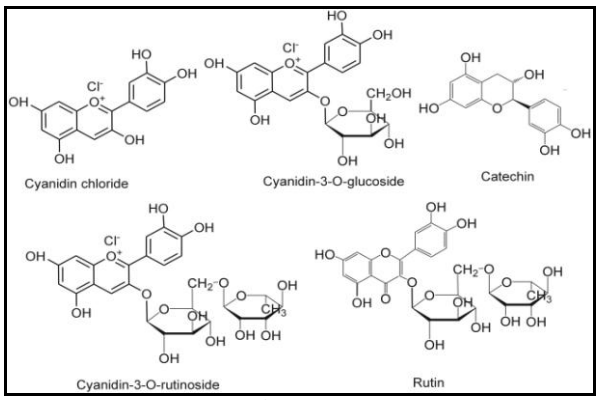


Figure 3. The structures of 5 phenolic compounds in *Begonia fimbristipula* Hance.

It is supported by our finding that a similar cyanidin compound (proanthocyanidin) exhibited stronger antioxidant ability in several assays (Table 1). In addition, rutin was demonstrated to be of radical-scavenging effects on •OH and O_2•$^-$ [22, 23]; cyanidin-3-O-rutinoside was regarded as one of the main resource of antioxidant ability in black rice [24]; catechin, however, could also effectively scavenge •OH and O_2•$^-$ radicals [25].

In conclusion, as a tea or functional vegetable in southeast China, *Begonia fimbristipula* Hance can effectively protect against hydroxyl-induced DNA damage. One mechanism of protective effect may be radical-scavenging which is via donating hydrogen atom (H•), donating electron (e). Its antioxidant ability may be mainly attributed to the existence of total phenols, especially cyanidin-3-O-glucoside.

COMPETING INTERESTS

None to declare

REFERENCES

1. Bhattacharjee S, Deterding LJ, Chatterjee S, Jiang J, Ehrenshaft M, Lardinois O, Ramirez DC, Tomer KB, Mason RP. Site-specific radical formation in DNA induced by Cu(II)-H_2O_2 oxidizing system, using ESR, immuno-spin trapping, LC-MS, and MS/MS. Free Radic Biol Med 2011; 50:1536-45.

2. The Editorial Board of Chinese Materia Medica. Chinese Materia Medica. Science & Technology Publishers, Shanghai, PR China, pp 493-495, 2000.

3. Huang WG, Zheng SY, Ye DM, Wang SY, Li XY, Wu QR, Yang YM, He JY, Yi Q. Protective effect against ionizing radiation damage from extract of *Begonia fimbristipula*. J Pract Med 2012; 28:1064-6.

4. Wang HS, Cao YM, Li GH, Lin MY, Guan AP, Deng LL. Effects of extract of *Begonia fimbristipula* on the development of diabetic nephrophathy rats. Chin J Biochem Pharm 2012; 33:272-7.

5. Li XC, Mai W, Wang L, Han W. A Hydroxyl-scavenging assay based on DNA damage *in vitro*. Anal Biochem 2013; 438:29-31.

6. Li XC. Improved pyrogallol autoxidation method: a reliable and cheap superoxide-scavenging assay suitable for all antioxidants. J Agric Food Chem 2012; 60:6418-24.

7. Li XC, Xu M, Chen D. Protective effect against hydroxyl-induced DNA damage and antioxidant activity of *Radix bupleuri in vitro*. Spatula DD 2012; 2:219-27.

8. Li XC, Zheng Y**,** Chen D. Protective efect against hydroxyl-induced DNA damage and antioxidant activity of *Radix codonopsis*. J Intercult Ethnopharmacol 2013; 2:1-8.

9. Oyaizu M. Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. Jpn J Nutr 1986; 44:307-15.

10. Li XC, Wang XZ, Chen DF, Chen SZ. Antioxidant activity and mechanism of protocatechuic acid *in vitro*. Funct Foods Health Dis 2011; 7:232-44.

11. Li XC, Wu XT, Huang L. Correlation between antioxidant activities and phenolic contents of Radix *Angelicae sinensis* (Danggui). Molecules 2009; 14:5349-61.

12. Dizdaroglu M, Jaruga P, Birincioglu M, Rodriguez H. Free radical-induced damage to DNA: mechanisms and measurement. Free Radic Biol Med 2002; 32:1102-15.

13. Jaruga P, Rozalski R, Jawien A, Migdalski A, Olinski R, Dizdaroglu M. DNA damage products (5'R)- and (5'S)-8,5'-cyclo-2'-deoxyadenosines as potential biomarkers in human urine for atherosclerosis. Biochemistry 2012; 51:1822-4.

14. Cheeseman KH, Beavis A, Esterbauer H. Hydroxyl-radical-induced iron-catalysed degradation of 2-deoxyribose. Quantitative determination of malondialdehyde. Biochem J 1988; 252:649-53.

15. Fang Y, Zheng R. Theory and Application of Free Radical Biology. Science Press, Beijing, PR China, 2002.

16. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT Food Sci Technol 1997; 30:609-15.

17. The Editorial Board of Chinese Materia Medica. Chinese Materia Medica. Science & Technology Publishers, Shanghai, PR China, pp 495-496, 2000

18. Tsimogiannis DI, Oreopoulou V. The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3′,4′-hydroxy substituted members. Innov Food Sci Emerg Technol 2006; 7:140-6.

19. Khanduja KL, Bhardwaj A. Stable free radical scavenging and antiperoxidative properties of resveratrol compared in vitro with some other bioflavonoids. Indian J Biochem Biophys 2003; 40:416-22.

20. Aliaga C, Lissi EA. Reaction of 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) derived radicals with hydroperoxides: kinetics and mechanism. Int J Chem Kinet 1998; 30:565-70.

21. Tan XS, Wang J, Tang TX. Study on the quality standard of *Begonia fimbristipulata* Hance. J Guangdong Pharm Univ 2012; 28:415-8.

22. Huo Q, Sun YJ, Cui YM, Zhang Y. Study on the antioxidant effect of rutin and its esterification products. Med Plant 2010; 10:71-6.

23. Huang YQ, Wang C, Liu HL, Zhang XF, Yan JZ. Research on the antioxidant ability of rutin, quercetin and TBHQ on pecan oil. Cereals Oils Process 2010; 4:13-5.

24. Wang JT, Yang M. The relationship between the chemical structures and its antioxidant ability of black rice proanthocyanidins. J Chongqing Unic Arts Sci 2007; 26:59-61.

25. Zhao WH, Deng ZY, Fan YW, Li J, Ruan Z. Research on antioxidant properties of (+)-catechin *in vitro*. Food Sci Technol 2009; 34:278-82.

 DOI 10.5455/oams.210713.or.049