

Full Length Research Paper

Antioxidant properties of banana flower of two cultivars in China using 2,2-diphenyl-1-picrylhydrazyl (DPPH,) reducing power, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate (ABTS) and inhibition of lipid peroxidation assays

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In this study, the antioxidant properties of banana flower extracts (cvs. Baxijiao (AAA) and Paradisiaca (AAB)) were analysed by using several biochemical assays which include 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate (ABTS) radical scavenging activities and inhibition of lipid peroxidation in egg lecithin through the formation of thiobarbituric acid-reactive substances (TBARS). These assays have been extensively studied and generally accepted as models to characterize peroxidative damage in biomembranes. In the present study, the EC₅₀ values were calculated using each method as listed above was used to compare the antioxidant efficiency of each banana flower extract. The phenol, flavonoid, vitamin E and saponin contents were also analyzed. Baxijiao flower extract revealed better antioxidant properties by presenting much lower EC₅₀ values, particularly for reducing power. In addition, antioxidant concentrations (polyphenols and flavonoids) were found higher in this flower sample than those in the Paradisiaca sample. The results suggested that the Baxijiao flower could be a better resource either as a dietary supplement or as a food additive than the later one.

Key words: Banana flower, antioxidant, scavenging effects, peroxidation.

INTRODUCTION

It is well known that herbal medicines can enhance human health as it has been recognized by the World Health Organization (WHO, 2002). As it has been well accepted, free radicals are a product of normal metabolism which causes molecular transformations and gene mutations in many types of organisms. Oxygen is essential for life, but its metabolites are highly toxic

(Gutteridge, 1993). Reactive oxygen species (ROS) are responsible in many cell disorders and the development of many diseases including atherosclerosis, cataracts chronic inflammation and neurodegenerative disease (Gutteridge, 1993). In healthy individuals, free radical production is continuously balanced by natural antioxidative defence systems. ROS and free radicals cause the deterioration of foods and as inducers of lipid peroxidation (Rechner et al., 2002). Antioxidants can delay or inhibit the oxidation of an oxidizable substrate in a chain reaction which are very important in the enhancing of human resistant in many diseases (Halliwell et al., 1992).

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Synthetic antioxidants are being restricted because of their toxic and carcinogenic effects. Thereby, interest in finding natural antioxidants, without undesirable side effects, has been increasing greatly (Rechner et al., 2002).

Banana (*Musa accminata* Colla) is a typical climac-teric fruit and mainly grows in tropical and subtropical regions. In China, lots of banana flowers are produced in Hainan annually, which have only been used as organic material and fertilizers up to date (Yang et al., 2003). According to Sheng et al. (2010), Banana flowers have tremendous nutritional value and healthy effect. These have been supported by Oliveira et al. (2006) who found that fatty acids and sterols were the major families of the lipophilic components in the floral stalk of "Dwarf Cavendish" banana. The chloroform, water and ethanol extract of *Musa sapientum* flowers were found to exhibit hypoglycaemic activities in alloxan diabetic rat (Dhanabal et al., 2005; Grover et al., 2002; Pari and Umamaheswari, 2000). Studies on the contents of vitamin C, tannin, myoinositol phosphates and alpha tocopherol in *M. sapientum* flower have been reported (Somsu et al., 2008). Ngamsaeng et al. (2006) investigated concentrations of crude saponin in *M. sapientum* flower and the relationship with the fermentation end-products. The above results can well explain that banana flowers have been consumed as food additives in many Asian countries such as Sri Lanka, Indonesia and Thailand (Wickramarachchi and Ranamukhaarachchi, 2005). However, there are limited reports regarding the flower antioxidant activities.

In our previous study, we have found that banana flowers are a good source of nutrients (Sheng et al., 2010). We assume that banana flowers are rich in antioxidant compounds. Therefore, the objective of this study was to evaluate the antioxidant activities of banana flowers extracted at two typical cultivars grown in Hainan, China (cvs. Baxijiao (AAA) and Paradisiaca (AAB)) using different biochemical assays. They were DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging activity, power of reduction, ABTS (2, 2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate)) radical scavenging activities, and lipid peroxidation inhibition in egg lecithin through the formation of thiobarbituric acid-reactive substances (TBARS).

MATERIALS AND METHODS

Sample preparation

Musa spp. 'Baxijiao' and 'Paradisiaca', the most popular and accessible banana flowers in Hainan, were selected in this study. The banana flowers used were grown in the experimental field of Haikou experimental station, The Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China, from January to December in 2008. The plants were managed as the commercial practices with standard fertilization and culture management. Mature banana plants from four plots, approximately 30 square meters per plot, were collected, and then the flowers were manually separated from the plant. After flowers were

collected, the samples were thoroughly washed in running water, cut into small pieces, dried overnight in an air dryer at 40 °C, ground to a particle size of 40 meshes, packed in black polyethylene bags, and then, stored at -20 °C prior to further analysis.

Chemicals and reagents

Folin-Ciocalteu reagent, gallic acid and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Company (St. Louis, MO). All reagents were analytical grade and met the basic requirements of above standard analysis according to each method.

Vitamin E

The content of vitamin E was determined by high performance liquid chromatography (HPLC, Shimadzu Co., Kyoto, Japan) as described by Ahn et al. (1995). Total amount of vitamin E was the sum of $\alpha + \gamma + \delta$ vitamin E.

Flavonoid concentration

The determination of flavonoid was performed according to the colorimetric assay of Kim et al. (2003). Distilled water (4 ml) was added to 1 ml of diisopropyl fluorophosphate extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately after, the volume of reaction mixture was filled upto 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g sample.

Total saponin

The total saponin content was determined according to the spectrophotometric assay described by Baccou et al. (1977). 0.5 g of ground banana flower samples were weighed into a screw-capped centrifuge tube, and 10 ml of 80% aqueous methanol added. The tubes were tightly capped and stirred overnight using a magnetic stirrer. The sample tubes were centrifuged at 3000 g for 10 min at room temperature and the supernatants were collected in 25 ml measuring flasks. The residues were washed twice with 5 ml of 80% aqueous methanol, followed by centrifugation, and the supernatants were collected in volumetric flasks. The final volume was made to 25 ml with 80% aqueous methanol. Aliquot samples from the flasks were used for saponin determination. The results are expressed as diosgenin equivalents from a standard curve of different concentrations of diosgenin in 80% aqueous methanol.

Extraction preparation

The samples (typically 5 g) were extracted by stirring with 50 ml of ethanol at 75 °C for 2.5 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 50 ml portions of methanol. The combined ethanol extracts were evaporated at 40 °C to dryness and redissolved in 70% ethanol at a concentration of 5.0 mg/ml and analyzed for their contents of polyphenols, DPPH radical scavenging activity, reducing power, 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) radical scavenging activities and inhibitory effect on lipid peroxidation.

Total phenol content

The total phenol content of extracts was determined by the Folin-Ciocalteu colorimetric method according to Singleton et al. (1999). Briefly, 1 ml of the extract solution was mixed with the Folin-Ciocalteu reagent (1 ml) and 7.5% Na₂CO₃ (3 ml). After 1 h of incubation at room temperature, the absorbance was measured against water at 760 nm (UV-2450 spectrophotometer). Gallic acid was used for establishing the standard curve (0 - 50 mg/l; $y = 0.1419x + 0.0079$; $R^2 = 0.9994$) and the results were expressed as mg of gallic acid equivalents/g of extract.

Free radical scavenging activity

DPPH radical scavenging was monitored according to the method of Yen and Chen (1995) with minor modification. Various concentrations of flower extracts (1 ml) were mixed with 4 ml of 70% ethanol solution containing DPPH radicals (40 µg / ml). The mixture was shaken vigorously and left to stand for 15 min in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation.

$$\% \text{ RSA} = [(A_{\text{control}} - A_s) / A_{\text{control}}] \times 100$$

Where, A_{control} is the absorbance of the control (solution to which no antioxidant was added) and A_s is the absorbance of the extract solution. The extract concentration providing 50% of free radical scavenging activity (EC_{50}) was calculated from the graph of radical scavenging activity (RSA) percentage against extract concentration. Gallic acid was used as standards.

Reducing power

The reducing power of the extracts was evaluated by the method of Oyaizu (1986). Various concentrations of the extracts (0.2 ml) were mixed with 1.0 ml of 200 mM sodium phosphate buffer (pH 6.6) and 1.0 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min. After 1.0 ml of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 4000 rpm for 10 min. The upper layer (2 ml) was mixed with 2 ml of deionised water and 0.4 ml of 0.1% of ferric chloride and the absorbance was measured spectrophotometrically at 700 nm (Barros et al., 2007). Control was prepared using distilled water instead of extract. The values were presented as the means of triplicate analyses. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. Gallic acid was used as a standard.

ABTS radical scavenging activities

The ABTS, radical cation decolorization assay was carried out, using an improved ABTS decolorization assay of Re et al. (1999). ABTS^{•+} was generated by oxidation of ABTS with potassium persulphate. ABTS was dissolved in deionized water at approximately 7 mM concentration, and potassium persulphate was added to give an approximate concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12–16 h) in the dark prior to use, and the ABTS^{•+} solution was diluted with ethanol, to an absorbance of 0.700 ± 0.020 at 734 nm. After addition of 3 ml of diluted ABTS solution (Absorbance at 734 nm = 0.700 ± 0.020) into 100 µl of plant extracts, the absorbency was measured at ambient temperature 10 min thereafter. All measurements were carried out triplicately. Gallic acid was used as a standard.

Inhibitory effect on lipid peroxidation

Liposomes were prepared according to the method of Cervato et al. (2000). Egg lecithin (20 mg) was dispersed in phosphate buffer (5.0 ml, 0.05 M, and pH 7.4) and sonicated in a sonicator for 30 min under N₂ atmosphere in an ice-cold water bath. About 0.1 ml of plant extracts was mixed with liposomes (0.1 ml), 50 mM FeSO₃ (0.05 ml) and 0.05 M phosphate buffer (3.0 ml, pH 7.4). The reaction mixture was incubated at 37°C for 40 min. After incubation, 1 ml trichloroacetic acid (10 %) and 1 ml thiobarbituric acid (0.4 %) were added to the mixture, and the mixture was incubated at 100°C for 15 min. The extent of oxidation of liposomes was subsequently determined by measuring the thiobarbituric acid-reactive substances (TBARS). The absorbance of the supernatant was measured spectrophotometrically at 532 nm. The result was expressed as inhibition in relation to a control test. Gallic acid was used as standard.

Statistical analysis

Triplicate analyses were conducted for each sample. The experimental data were expressed as mean ± standard deviations of three separate determinations. One-way analysis of variance (ANOVA) was carried out on the experimental results using flowers species as an independent variable. The significance of differences between means were compared by Tukey's multiple tests at $p < 0.05$. All calculations were performed using an ANOVA package from statistical analysis systems (SAS, version 8.0).

RESULTS AND DISCUSSION

Yield and content of antioxidant compounds

The extracted yield of these banana flowers are listed in Table 1 and expressed in terms of the solid content in the dried product per soluble solid. Yields of Baxijiao and Paradisiaca flower are $20.52 \pm 0.22\%$ and $15.57 \pm 0.36\%$, respectively. Even through the low yield productions, the antioxidants were found in both samples, indicating the effective extraction of these antioxidant compounds. Phenolics are plant secondary metabolites which are very important in chelating redox-active metal ions, inactivating lipid free radical chains, and preventing hydroperoxide conversions into reactive oxyradicals as they have been generally recognized. Table 1 summarized the total phenolic compounds in fractions expressed as gallic acid equivalents (GAE), which varied between 6.58 ± 0.03 mg and 10.20 ± 0.03 mg/g at a dry weight basis.

The flower sample of Baxijiao showed a higher phenolic content (10.02 ± 0.03 mg/g) than samples extracted from the flower of Paradisiaca. The content of total flavonoids expressed as catechin equivalents, varied from 5.9 ± 0.75 to 5.27 ± 0.47 mg as catechin equivalent/100 g fraction. The current results have confirmed that higher flavonoids (5.27 to 5.90 mg/100 g) were presented in both banana flowers than in banana peels as it has previously been reported (Lima et al., 2008). It is suggested that banana flowers are good sources of flavonoids. Phenolic acids and flavonoids have been reported to be the main phytochemicals responsible for the antioxidant capacity of fruits and vegetables. Plant derived polyphenols

Table 1. Chemical composition of banana flowers in two cultivars (cvs Baxijiao and Paradisiaca).

| Component | Baxijiao | Paradisiaca |
|-----------------------------|-------------|-------------|
| Total phenol (mg/g) | 10.20±0.03a | 6.58±0.03b |
| Vitamin E (mg/kg) | 0.87±0.04a | 1.07±0.06b |
| Total Saponin (g/100 g) | 0.11±0.00a | 0.12±0.01a |
| Total Flavonoids (mg/100 g) | 5.90±0.75a | 5.27±0.47a |
| Extraction yield (%) | 20.52±0.22a | 15.57±0.36b |

Values are means ± standard deviations of triplicate determinations; values in the same column followed by different letters are significantly different according to ANOVA at $P < 0.05$.

Table 2. EC₅₀ values (µg/ml) and anti-radical power obtained in the antioxidant assays of areca extracts

| Assay | EC ₅₀ of Baxijiao | EC ₅₀ of Paradisiaca | EC ₅₀ of Gallic acid |
|---|------------------------------|---------------------------------|---------------------------------|
| DPPH radical scavenging activity | 4.84 | 5.84 | 3.69 |
| Reducing power | 2.12 | 2.41 | 7.91 |
| ABTS radical scavenging activities | 4.16 | 4.09 | 2.46 |
| Inhibitory effect on lipid peroxidation | 5.45 | 5.98 | 2.88 |

display characteristic inhibitory patterns toward the oxidative reaction *in vitro* and *in vivo* (Bahramikia et al., 2009).

The concentration of vitamin E in Baxijiao flower was found to be significantly lower than Paradisiaca flower ($p < 0.05$) (Table 1). The value found in banana flowers in this study was higher than those reported in other tropical plants (Ching and Mohamed, 2001). The saponin concentration of samples, which ranged from 0.11 - 0.12% was lower than the previous result which was 1.3% obtained in banana leaves (Ngamsaeng et al., 2006). No significant difference was found between two cultivars regarding the saponin concentrations (Table 1).

Free radical scavenging activity

DPPH is a stable organic nitrogen radical and free radical compound with a purple colour which change into a stable yellow compound in reacting with an antioxidant. In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by the antioxidant (Duh, 1998). The assay of the scavenging of DPPH radical is widely used to evaluate the antioxidant capacity of extracts from different plant materials (Amarowicz et al., 2004).

The DPPH free radical scavenging activities and EC₅₀ values of banana flower extracts and gallic acid at various concentrations of the extract are presented in Table 2 and Figure 1, respectively. The addition of the flower extract into the DPPH solution caused a rapid decrease in absorbance at 517 nm indicating the excellent scavenging capacity of the flower extracts. The extracts posses-

sed substantial dose-dependent antioxidant activity. With regard to EC₅₀ values (the concentration of antioxidant required to achieve absorbance equal to 50% that of a control containing no antioxidants), Baxijiao (EC₅₀ of = 4.84µg/ml) and gallic acid (EC₅₀ of =3.69µg/ml) were higher in radical scavenging abilities in comparison to Paradisiaca (EC₅₀ of = 5.84 µg /ml) sample. Exposure to proton radical scavengers is known to significantly reduce the level of DPPH (Yamaguchi et al., 1998). Therefore, free radical-scavenging activity has a marked impact on the phenolic composition of the sample.

The antioxidant activities of plant materials strongly correlates with their content of the phenolic and flavonoid compounds (Velioglu et al., 1998). In the present study, the flavonoid content was higher than the total phenolic content in the ethanol extract of banana flower samples (Table 1). Therefore, the higher free-radical-scavenging activities of the ethanol extract of banana flower samples may be due to the higher amounts of flavonoid compounds in those samples.

Reducing power

According to the Oyaizu's (1986) method the reduction of Fe³⁺ to Fe²⁺ was determined by measuring absorbance of the Perl's Prussian blue complex. This method was based on the reduction of (Fe³⁺) ferricyanide in stoichiometric excess relative to the antioxidants. Fig. 2 shows the reducing power of banana flower extracts as a function of their concentrations based on the ability to reduce ferric (Fe³⁺) to ferrous (Fe²⁺) ion through the donation of an electron. The resulting ferrous ion (Fe²⁺)

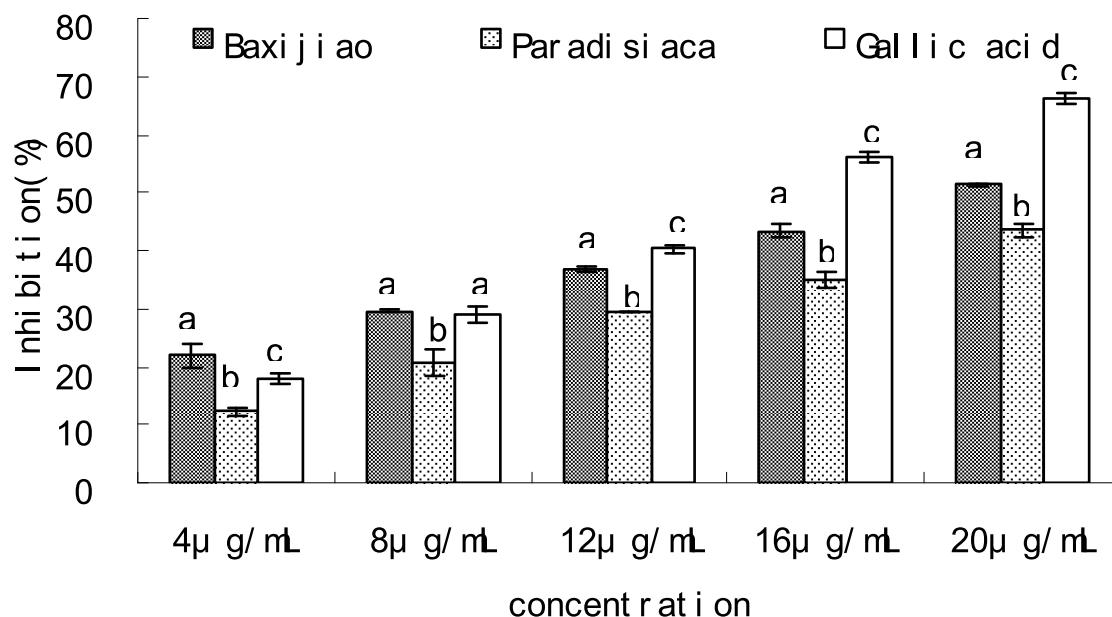


Figure 1. DPPH radical scavenging activity under different concentrations in two flower extracts and gallic standard sample. Different letters at each concentration mean significantly different among the three columns/samples at $p < 0.05$.

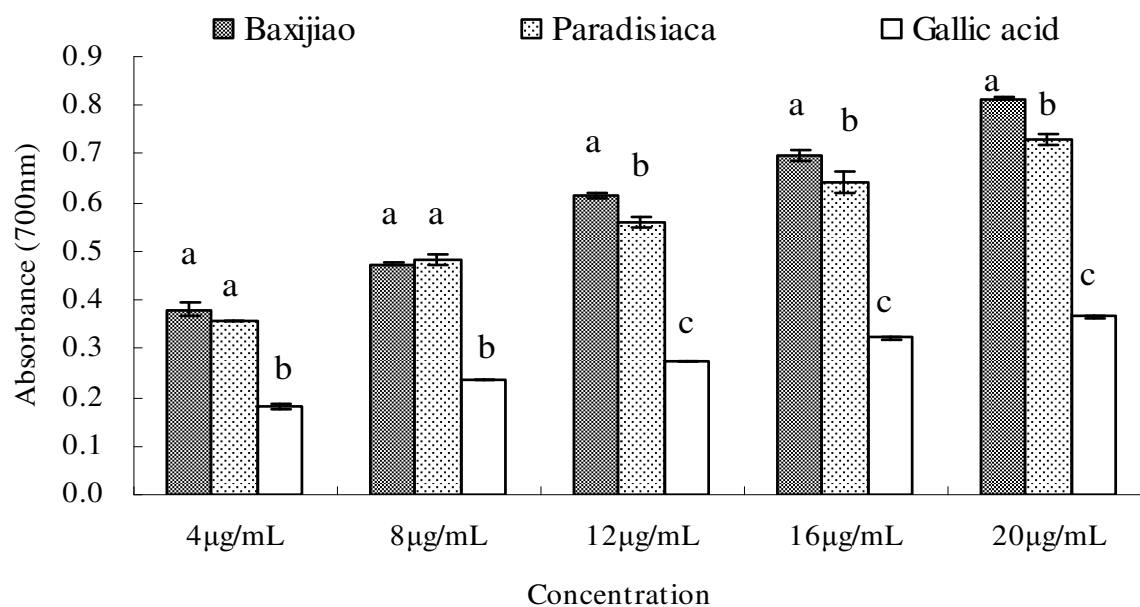


Figure 2. Reducing power under different concentrations in two flower extracts and gallic standard sample. Different letters at each concentration mean significantly different among the three columns/samples at $p < 0.05$.

was measured at 700 nm. This assay just indicates how easily a given antioxidant donates electrons to reactive free radicals species, thus promoting the termination of free radical chain reactions. The ability of the antioxidant to reduce Fe^{3+} to its more active Fe^{2+} form might also be an indicator of its ability to act as a preoxidant in the system.

The reducing power of banana flower extracts increased with concentration and possessed substantial dose-dependent antioxidant activity. The reducing power of the extract was excellent and significantly ($p < 0.05$) higher than that of the gallic acid. At 20 $\mu\text{g}/\text{ml}$, the reducing power was higher than 0.7 and in the order as follows: Baxijiao > Paradisiaca > Gallic acid. At 4 $\mu\text{g}/\text{ml}$,

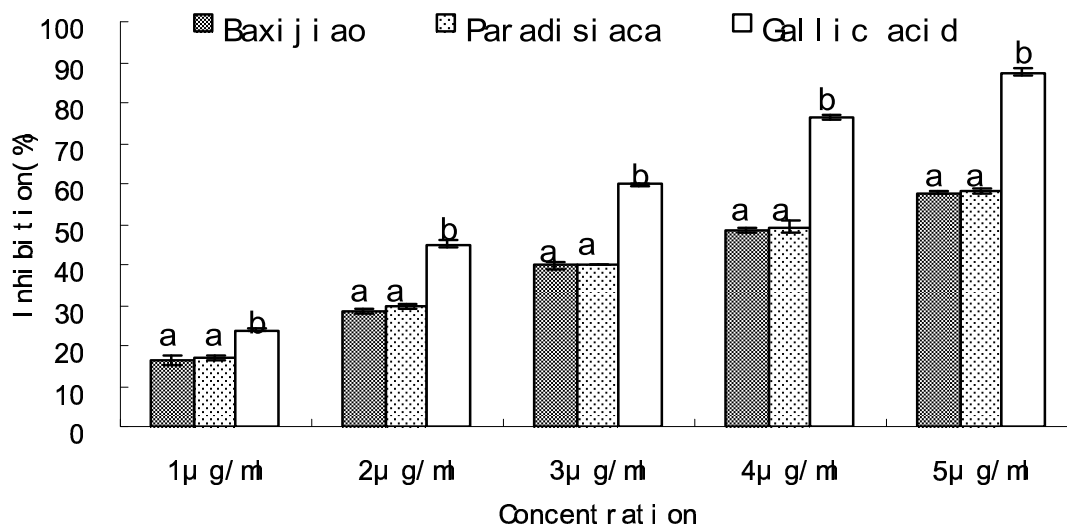


Figure 3. ABTS radical scavenging activities under different concentrations in two flower extracts and gallic standard sample. Different letters at each concentration mean significantly different among the three columns/sample at $p < 0.05$.

the reducing power of the ethanol extracts from banana flowers were between 0.36 ± 0.01 – 0.38 ± 0.01 . Reducing power of Gallic acid at 20 and $4 \mu\text{g/ml}$ were only 0.37 ± 0.02 and 0.18 ± 0.01 , respectively. The results were in agreement with the EC_{50} of extracts and Gallic acid. Ethanol extracts from Paradisiaca showed slightly lower reducing power values (Fig. 2) than those from the Baxijiao flower sample (Fig. 1). In general the flowers extracts have been proved to be better sources of antioxidants than the Gallic acid.

ABTS radical scavenging activities

ABTS assay was based on the antioxidant ability to react with $ABTS^+$ generated in the system. This method is widely used to evaluate antioxidant activities in foods and biological systems (Meyer et al., 2001). The advantages of this radical are its water-solubility and high absorption coefficient at long wavelengths, allowing the determination of its rate of consumption with minimal interferences (Campos and Lissi, 1997). In addition, scavenging activity of the ABTS radical is less susceptible to steric hindrance when bulky antioxidants are used. The $ABTS^+$ scavenging ability of tested compounds are listed in Table 2. Based on EC_{50} values, the potency of $ABTS^+$ was in a decreasing order as follows: Gallic acid > Paradisiaca > Baxijiao. As it has been shown in Figure 3, the $ABTS^+$ scavenging activities of banana flower extracts were lower than that of the gallic acid.

Inhibitory effect on lipid peroxidation

Since lipids are very susceptible to lipid peroxidation, we

also tested the ability of the samples to prevent the peroxidation of polyunsaturated fatty acids induced by hydroxyl radical. Inhibition of lipid peroxidation was evaluated using thiobarbituric acid-reactive substances. This is a highly sensitive method, and the results depend on the centrifuging degree in removing the precipitated protein. Otherwise, this will lead to erroneous absorbance results as can be easily understood from Figure 4.

The capacity of inhibition of lipid peroxidation is proportional to the extract concentration. This method permitted the achievement of very high inhibition percentages at extremely low concentrations. Table 2 shows antioxidant activity with EC_{50} values of Baxijiao, Paradisiaca flower extracts and gallic acid measured by different biochemical assays. The extract of Baxijiao flowers revealed better antioxidant properties in two cultivar extracted by the DPPH method, reducing power assay, ABTS and inhibitory effect on lipid peroxidation. The obtained results were in agreement with total phenol and flavonoid contents determined for each sample and shown in Table 1. The EC_{50} values of Baxijiao flower samples obtained for reducing power were the best compared to those for DPPH radicals, ABTS and inhibitory effect on lipid peroxidation.

Conclusion

This study has comprehensively investigated the antioxidant properties of banana flowers of two commercial cultivars, that is Baxijiao and Paradisiaca grown in Hainan, China. The results indicate that these banana flowers are good sources of antioxidants including phenolics and flavonoids. Generally, Baxijiao flower presents higher

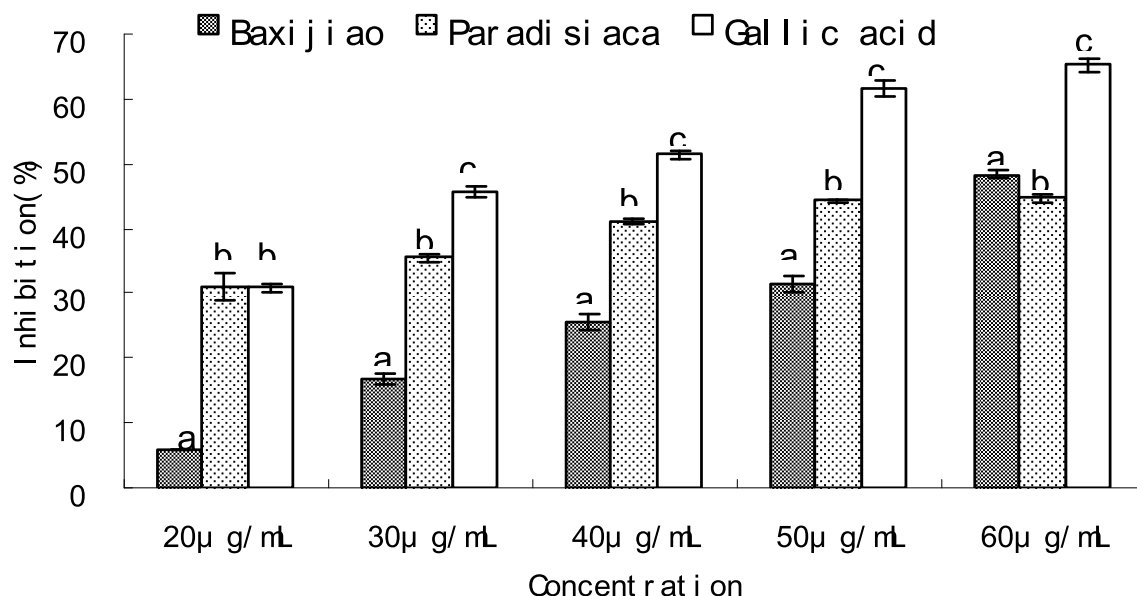


Figure 4. Inhibitory effect on lipid peroxidation under different concentrations in two flower extracts and gallic standard sample. Different letters at each concentration mean significantly different among the three columns/sample at $p < 0.05$.

antioxidant activities in comparison to the Paradisiaca flower sample, using the assays as discussed above. It suggests that banana flowers should be considered as one of the most active substance which may positively affect human-health and can be used in food industry as an additive. Therefore, further study is required in identification and purification of antioxidant compounds in banana flowers from scientific and commercial standpoint.

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Abbreviations

ABTS, 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate); **TBARS**, thiobarbituric acid-reactive substances; **ROS**, reactive oxygen species; **DPPH**, 1,1-diphenyl-2-picrylhydrazyl; **HPLC**, high performance liquid chromatography; **CEQ**, catechin equivalents; **RSA**, radical-scavenging activity; **GAE**, gallic acid equivalents.

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