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ORIGINAL ARTICLE

# Free Radical-scavenging Activities and Cytoprotective Effect of Polyphenol-rich ethyl acetate Fraction of Guava (*Psidium cattleianum*) Leaves on $H_2O_2$-treated HepG2 Cell

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**Abstract** Total phenolic contents of different fractions of the *Psidium cattleianum* leaf extract and their antioxidant capacity against several free radicals were examined. Protective effect of the ethyl acetate fraction (EAF) on $H_2O_2$-induced DNA damage in HepG2 cells were also evaluated, and the phytochemical profile of EAF was analyzed using tandem mass spectrometry. EAF derived from the 80% methanol extract of the leaf contained a remarkable amount of polyphenol and showed high levels of DPPH and alkyl radical scavenging activity, promoted cell viability, and protected against $H_2O_2$-induced DNA damage in HepG2 cells. Phytochemical analysis revealed that the major components in the EAF included quercetin monoglycoside, phloridizin, quercetin 3-diglycoside, quercetin-3-glucuronide, 2,6-dihydroxy-3,5-dimethyl-4-glucopyranosyl-benzophenone, phenolic acid, guaijaverin, and naringin. The present study suggests possible synergistic or competitive antioxidant action of the major compounds of cattley guava leaf on $H_2O_2$-induced DNA damage in HepG2 cells. These results indicate that the ethyl acetate fraction of the guava leaf could be used as a potential source of natural antioxidants, and these findings will facilitate the utilization of guava leaf as a source of functional food.

**Keywords** antioxidant · guava leaf · free radical · HepG2 · polyphenol

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## Introduction

Reactive oxygen species (ROS) and other free radicals are produced during the normal cell metabolism, a necessary and normal process that provides important physiological functions (Cui et al., 2004; Valko et al., 2007; Oakely et al., 2009). The production of ROS and other free radicals is normally compensated by an elaborate endogenous antioxidant system. However, due to many environmental, lifestyle, and pathological factors, excess radicals can accumulate in cells, resulting in oxidative stress. At high concentrations, ROS are deleterious due to their high reactivity, as they may attack biological macromolecules inducing oxidation and causing membrane damage, enzyme inactivation, and DNA damage. Oxidative stress has been recognized to be involved in the etiology of several diseases, and antioxidants are necessary to cure these diseases. However, the synthetic antioxidants could be unsafe, natural antioxidants have been proposed and utilized as therapeutic agents to counteract such diseases.

It is believed that fruits and vegetables provide protection against several health disorders, because they are rich sources of antioxidants, which scavenge free radicals and thereby reduce the incidence of degenerative pathologies (Wootton-Beard and Ryan, 2011). A plethora of data from previous studies indicated that a number of plant products including polyphenols, flavonoids, terpenes, and various plant extracts exert an antioxidant action; they have been reported to inhibit the propagation of free radical reactions and to protect the human body from disease (Liu and Ng, 2000). Polyphenols scavenge $H_2O_2$ and reduce the amount of DNA oxidative damage or rapidly repair the transient DNA damage resulting from $H_2O_2$ attack before enzymic repair initiation (Tan et al., 2009).

*Psidium cattleianum*, commonly known as guava, is widely grown in subtropical climatic region all over the world as subtropical plant belonging to Myrtaceae. It has been claimed to be effective in the treatment of diarrhea, dysentery, acute gastrointestinal inflammation, and diabetes (Oh et al., 2005). We previously reported that the chloroform fraction of leaf extract of cattley guava (*P. cattleianum)* from Jeju, showed antiproliferative activity via induction of apoptosis in human gastric cancer cells (Moon et al., 2011). Guava leaves have also been shown to have hepatoprotective effects (Roy et al., 2006). However, the antioxidant properties of cattley guava leaf have not been studied in detail, and the effect of this guava leaf extracts on DNA protection is still unknown, whereas the genus *Psidium* has been investigated particularly for antioxidant properties (Gutierrez et al., 2008).

HepG2 cells, a human hepatoma cell line, are considered a good model to study *in vitro* cytotoxicity to the liver, because they retain many of the characterized functions of normal human hepatocytes (Knasmuller et al., 1998). In particular, HepG2 cells retain the activity of many phase I, phase II, and antioxidant enzymes ensuring that they constitute a good tool to study cytoprotective, genotoxic, and antigenotoxic effects of compounds (Knasmuller et al., 2004; Mersch-Sundermann et al., 2004). Recent studies on cytoprotection by natural antioxidants in HepG2 cells have increasingly been using $H_2O_2$ or tert-butyl hydroperoxide, an organic hydroperoxide, as the toxic agent (Kinjo et al., 2003; Lee et al., 2005a; Lee et al., 2005b; Alia et al., 2006). The present study was carried out to measure the total phenolic content of various fractions of leaf extract, their antioxidant capacity assessed against several free radicals, the protective effect of ethyl acetate fraction (EAF) on $H_2O_2$-induced DNA damage in HepG2 cells evaluated, and the phytochemicals present in EAF investigated by the modern tendem mass spectrometry. Understanding the mechanism of action of *P. cattleianum* leaf extract on protective effects in HepG2 cells from oxidative stress will facilitate the utilization of its leaves as a source of functional food.

## Materials and Methods

**Chemicals.** High-performance liquid chromatography (HPLC)-grade acetonitrile, water, and trifluoroacetic acid (TFA) were purchased from Merck (Germany) and Fisher Scientific, Ltd. (USA). Folin-Ciocalteu phenol reagent, rutin, gallic acid, hydrogen peroxide, ferrous sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH), and α-(4-pyridyl-1-oxide)-*N*-*t*-butylnitrone (4-POBN) were purchased from Sigma Chemical Co. (USA). Dulbecco's modified Eagle's medium (DMEM), trypsin/(EDTA), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Invitrogen Life Technologies, Inc. (USA). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide, RNase A, and caspase activity assay kits were purchased from Sigma Chemical Co. (USA). A BCA protein assay kit was purchased from Pierce (USA). All other reagents used were of analytical grade.

**Plant material.** Cattley guava (*P. cattleianum*) leaves were collected from a farmer's field near the National Institute of Subtropical Agriculture in Jeju province, Korea, and a voucher specimen (NO: 109/08) was deposited in the herbarium of the Subtropical Research Institute of Jeju National University for further reference.

**Extract and sample preparation.** Air-dried leaves (1200 g) were pulverized using a milling machine and extracted with 80% methanol by stirring for 3 days at room temperature. The extract was filtered, concentrated with a vacuum rotary evaporator under reduced pressure at 40°C, and lyophilized to afford a crude methanol extract (27.0 g). The dried methanol extract (20.0 g) was then suspended in water (500 mL) and further fractionated by additional extraction with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol in a stepwise manner. Each fraction was lyophilized to obtain the powder of corresponding fractions of leaf extract, and its powder was dissolved in 200 mg/mL DMSO. The yield of ethyl acetate fraction (EAF) was 0.067% (0.8 g).

**Determination of total polyphenol.** Total polyphenol was determined according to the method of Cheung et al. (2003) with minor modifications. Sample aliquots (1.5 mL) were mixed with 0.5 mL of Folin-Ciocalteu phenol reagent. After 5 min, 1 mL of 10% (w/v) $Na_2CO_3$ was added to each reaction mixture. Reactions (30 min) were performed in the dark, and absorbance at 725 nm was then recorded using a UV 1800 spectrophotometer (Shimadzu, Japan). Results were expressed in terms of gallic acid equivalents (GAE) (mg GAE/g of dried sample). All analyses were performed in at least triplicate.

**Determination of free radical scavenging activity using ESR spectrometry.** The DPPH radical-scavenging activity was measured using a previously described method (Nanjo et al., 1996). A 30-μL aliquot of each sample diluted in phosphate buffered saline (PBS) was added to 30 μL of DPPH (60 μM) in ethanol. PBS without test sample was used as the control. Final concentration range was 6.25 to 25 μg/mL for each extract. After mixing vigorously for 10 s, solutions were transferred to 50-μL Teflon capillary tubes and inserted into the cavity of a JES-FA electron spin resonance

(ESR) spectrometer (JEOL, Japan). Supernatants were measured after 2 min. Measurement conditions were as follows: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW, gain $6.3\times10^5$, and temperature 297 K. The radical-scavenging activities of the pitaya extracts were calculated according to the following formula: Scavenging rate = $(h_0-h_x)/h_0\times100\%$, where $h_0$ and $h_x$ are the ESR signal intensities of samples containing and not containing extract, respectively. Hydroxyl radicals were generated by the Fenton reaction and reacted rapidly with nitrone spin trap DMPO. The resulting DMPO-OH adduct was detected by ESR (Rosen and Rauckman, 1984). The ESR spectrum was recorded 2.5 min after mixing with phosphate buffer solution (pH 7.4) supplemented with 20 μL of 0.3 M DMPO, 20 μL of 10 mM $FeSO_4$, and 20 μL of 10 mM $H_2O_2$ under the following conditions: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 1 mW, gain $6.3\times10^5$, and temperature 298 K. The final concentration range was 0.5 to 2 mg/mL for each extract. The radical-scavenging activities of the leaf extracts were calculated according to the following formula: Scavenging rate = $(h_0-h_x)/h_0\times100\%$, where $h_0$ and $h_x$ are the ESR signal intensities of samples containing and not containing extract, respectively. Alkyl radicals were generated by AAPH. Reaction mixtures containing 40 mM AAPH, 40 mM 4-POBN, and the indicated concentrations of tested samples diluted in PBS (pH 7.4) were incubated at 37°C in a water bath for 30 min (Hiramoto et al., 1993) and then transferred to 50-μL Teflon capillary tubes. The final concentration range was 12.5 to 50 μg/mL for each extract. The spin adduct was recorded using the ESR spectrometer. The following measurement conditions were used: central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 10 mW, gain $6.3\times10^5$, and temperature 298 K. The radical scavenging activities of the leaf extracts were calculated according to the following formula: Scavenging rate = $(h_0-h_x)/h_0\times100\%$, where $h_0$ and $h_x$ are the ESR signal intensities of samples containing and not containing extract, respectively.

**Cell culture.** HepG2, a human hepatocarcinoma cell line (obtained from the Korean Cell Line Bank, Korea) was cultured in DMEM containing 10% (v/v) heat-inactivated FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained in a humidified incubator at 37°C in 5% $CO_2$ atmosphere.

**Cytotoxicity assay.** To evaluate the ability of the extracts for protection of cultured cells from oxidant-induced cell death, the MTT colorimetric assay was performed to determine the cell viability (Hansen et al., 1989). HepG2 cells were cultured at $5\times10^4$ cells/mL in 96-well plates for 16 h, washed twice using PBS, and pretreated with EAF. After 1 h incubation, 200 μM of $H_2O_2$ solution were added to the wells, and the cells were re-incubated for 3 h. MTT reagent (5 mg/mL) was added to each well, and the plate was incubated at 37°C for additional 4 h. Subsequently, the medium was removed and the intracellular formazan product was dissolved in DMSO. Absorbance at 570 nm of the mixture was detected using microplate reader (Tecan, Austria). The percentage of cell viability was calculated based on the following formula: mean value of (control group−treated group/control group)×100%. All results were assessed in triplicate for each concentration.

**Determination of DNA damage (Comet assay).** The Comet assay was conducted according to the method of Tice et al. (2000) with slight modification. The cells in a 24-well plate were treated with the EAF, as described above. The cell suspension was mixed with 100 mL of 0.5% low-melting point agarose (LMPA) and added to slides pre-coated with 1.0% normal melting agarose. After solidification of the agarose, the slides were covered with additional 100 mL of 0.5% LMPA and then immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium laurylasarcosine, 1% Triton X-100, and 10% DMSO) at 4°C for 1 h. For electrophoresis of the DNA, an electric current of 25 V/300 mA was applied at 4°C for 20 min. The slides were washed three times with a neutralizing buffer (0.4 M Tris-HCl, pH 7.5) at 4°C for 5 min, and treated with ethanol for additional 5 min before staining with 50 mL ethidium bromide (20 mg/mL). The percentage of fluorescence in the DNA tail of each cell (100 cells from each of triplicate slides) on the ethidium bromide stained slides were measured by image analysis (Kinetic Imaging, Komet 5.0, UK) and fluorescence microscopy (LEICA DMLB, Germany).

**Analysis by HPLC-tandem mass spectrometry.** HPLC-PDA-ESI-MS analysis was conducted using a Thermo Scientific Surveyor HPLC and an LTQ linear ion trap mass spectrometer (Thermo Scientific Inc, Waltham, USA) with an electrospray ionization (ESI; Bruker Esquire 3000 plus) interface at 40°C, coupled with a photodiode-array detector (PDA) set at 280 nm. UV spectra were scanned from 200 to 700 nm. EAFs were dissolved in a mixture of acetonitrile/water (50/50, v/v), filtered through a PTFE 0.20-μm syringe filter (Advantec, Japan), and 2 μL was injected into the HPLC column. Chromatographic separation was performed on a Hypersil GOLD C18 column (100×2.1 mm; I.D, 1.9 μm) with eluent A ($H_2O$ plus 0.05% TFA) and eluent B (acetonitrile plus 0.05% TFA) at a flow rate of 200 μL/min according to the following gradient elution profile: 0–25 min, 90–40% A; 25–35 min, 40–2% A; 35–38 min, 2% A; 38–43 min, 2–40% A; and 43–45 min, 40% A.

ESI experiments were performed in both positive and negative modes. The settings were as follows: mass range measured *m/z* 100–1000; ion trap temperature, 325°C; Em, 5.0 kV; drying $N_2$, 10 mL/min; nebulizing $N_2$, 30 psi; sheath gas flow, 30 units; capillary voltage, 9 V; and collision gas, helium. Tentative structural identification was performed by mass-selection of the ion of interest with a selected ion chromatogram of the individual species. $MS^n$ spectra were obtained in auto $MS^2$ mode (the product ion of the base peak was selected automatically as a precursor ion for the next stage MS), which provided additional structural information. Instrument operation, and data were collected with commercially available Thermo Finnigan Xcalibur software (Finnigan Corp, USA).

**Statistical analysis.** All presented data were repeated at least three times. Values are expressed as means ± SD. Statistical analysis

was performed using analysis of variance. Values of $*p < 0.05$, $**p < 0.01$ were considered to be statistically significant.

## Results and Discussion

**Total polyphenol.** Plant phenolics have been reported to exhibit antioxidant activity due to the reactivity of the phenol moiety as well as the ability to scavenge free radicals via hydrogen donation or electron donation. It has been known that the amount of functional compounds recovered from raw plant materials depends on the method used for extraction. Present study showed that guava leaf fraction contained phenolic compound at different levels according to the polarity of solvent used in the extraction process, in the following order: Buthanol Fraction (BF) > Ethyl Acetate Fraction (EAF) > 80% Methanol Extract (ME) > Water Fraction (WF) > Chloroform Fraction (CF) > Hexane Fraction (HF) (Table 1). It is possible that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of the quantity and quality of the phenolic constituents of the extracts. Oxidation-reduction determines not only phenolic compounds but also other chemical components such as carotenoids, amino acids, sugars, and vitamin C. In light of the above limitations, it is perhaps more appropriate to use the term Folin-Ciocalteu index rather than total phenolics. Nevertheless, this widely used method provides a rapid and useful overall evaluation of the phenolic content of extracts. The phenolic compounds present in the guava leaves play an important role in antioxidant activity, directly through the reduction of oxidized intermediates in the chain reaction.

**Antioxidant activity.** Evaluating antioxidants in plants is still challenging from the analytical point-of-view, because they are always present as very complex mixtures. ESR provides a sensitive, direct, and accurate means of monitoring reactive species; thus, we used it to compare the DPPH, hydroxyl, and alkyl radical-scavenging abilities of the fractions of 80% methanol extracts of cattley guava leaf, using catechin as a standard.

**DPPH radical-scavenging activity.** DPPH is a stable free radical, whose level decreases significantly following exposure to proton radical scavengers. As shown in Fig. 1A, the EAF displayed the highest DPPH radical scavenging activity, followed by BF, ME, WF, HD and CF at different concentration. As expected, catechin also showed good antioxidant activity at all tested concentration. In recent report, the acetone extracts of *P. cattleianum* Sabine fruits showed higher antioxidant activity, which was correlated to high levels of phenolic compounds (Medina et al., 2011). Interestingly, EAF would appear to be more potential in this study during DPPH free radical-scavenging activity.

**Hydroxyl radical scavenging activity.** Plant-derived phenolic compounds are known to exert scavenging activity against hydroxyl radicals, and the oxidation products of phenolic compounds are aldehydes and dimers. Hydroxyl radicals generated in the $Fe^{2+}$/$H_2O_2$ system were trapped by a DMPO-forming spin adduct that was detected using an ESR spectrometer (Saito et al., 2008). No significant difference was observed among the tested fraction at various concentrations with EAF and BF being the top two fractions (Fig. 1B). Both EAF and BF exhibited reasonable activity and comparable with methanol fraction, because they contained high amounts of polyphenols. In addition, HF and CF also displayed good activity, even though they contained the lowest amounts of polyphenols. Due to high reactivity, hydroxyl radicals may not provide an accurate reflection of the ability of antioxidant molecules to confer oxidative protection when non-site-specific methods are used (Adebajo and Gesser, 2001). Nevertheless, our findings suggest that EAF and BF may offer some protection against hydroxyl radicals.

**Table 1** Total phenolic content from different fraction of *P. cattleianum* leaf extract

| Sample | Total polyphenol content (mg/g) Gallic acid |
|---|---|
| HF | 157.2±16.6 |
| CF | 166.1±17.1 |
| EAF | 318.3±44.1 |
| BF | 326.9±38.8 |
| WF | 227.3±29.3 |
| ME | 275.4±29.6 |

**Alkyl radical-scavenging activity.** Alkyl radicals are primary intermediates in many hydrocarbon reactions and can be easily detected by ESR (Kim et al., 2010). Similar to DPPH radical-scavenging activities, EAF and BF exhibited moderately higher radical-scavenging activities than the other fraction. Catechin displayed strong alkyl radical-scavenging activity at different concentrations (Fig. 2C). Although phenolic antioxidants are known to trap peroxy radicals to protect organic materials from oxidative degradation, the trapping of alkyl radicals is also important in preventing such degradation, because autooxidation involves both peroxy and alkyl radicals acting as chain carriers. On the basis of scavenging abilities, both EAF and BF of cattley guava leaf showed potential as a rich source of alkyl radical scavengers.

**Effects of EAF and BF fractions on $H_2O_2$-induced cytotoxicity.** The ROS ($H_2O_2$) has been reported to generate DNA damage, induce chromosomal aberrations, cause gene mutations, and break single-strand DNA. In the present study free radical-scavenging activities of both EAF and BF were found to be quite similar by using ESR spectrometry; thus the protective effects of EAF and BF on cell viability in $H_2O_2$-treated cells were compared. Fig. 2 shows the effect of pre-incubation with both fractions on HepG2 cell death caused by treatment of 200 μM $H_2O_2$. In our MTT sassy, exposure of cells to 200 μM $H_2O_2$ increased cell death by up to 50% and pre-incubation of cells with EAF inhibited the cell death induced by $H_2O_2$ for 1 h. At a concentration of 100 μg/mL, the cell viability was restored to about 80% by pre-treatment with EAF, whereas the cell viability of the cells treat with the BF exhibited similar level of cytotoxicity to that of the cells treated

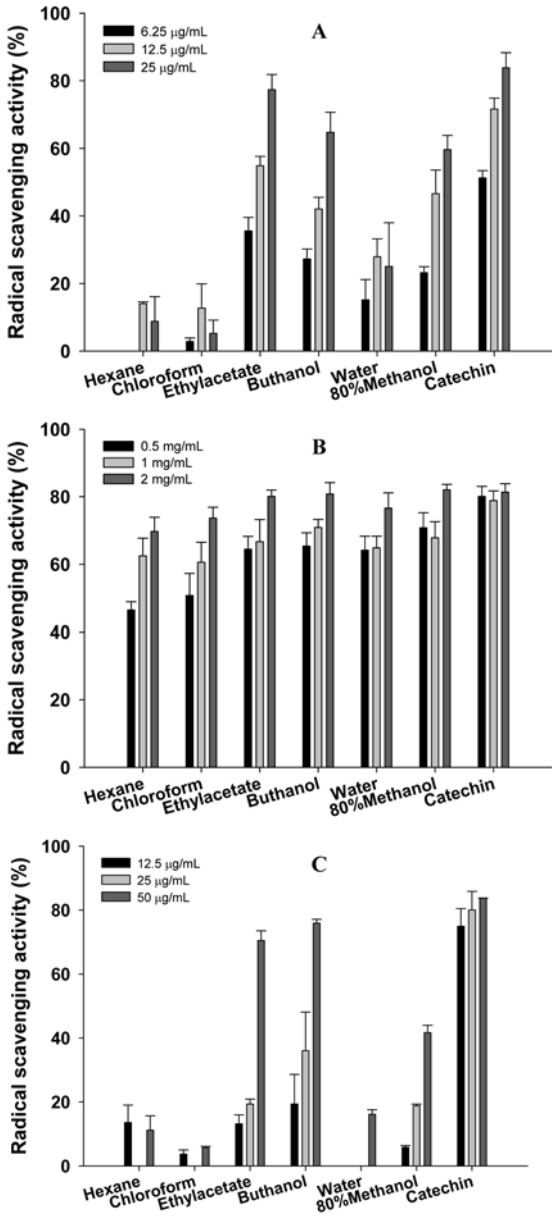


**Fig. 1** Radical scavenging activity of various fractions of *P. cattleianum* leaf extract. (A) DPPH, (B) hydroxyl, and (C) alkyl radical. All fractions were added at 6.25–25 μg/mL (A, C), 0.5–2 mg/mL (B). Catechin was used positive control. Values are means ± SD (n =3).

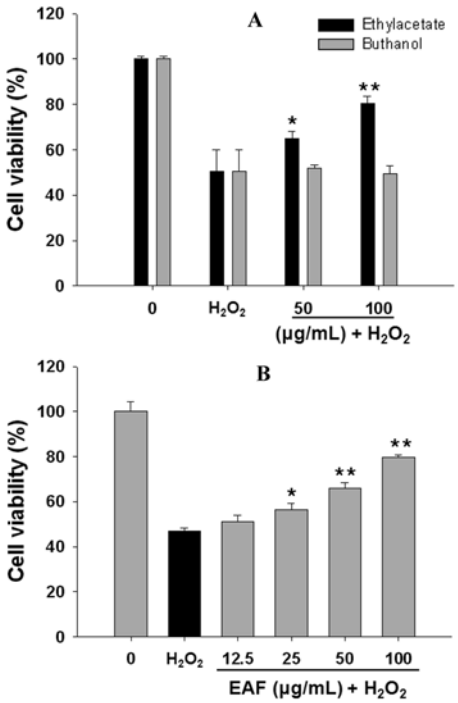


**Fig. 2** Protective effects of EAF of *P. cattleianum* leaf. (A) HepG2 cell culture were treated for 1 h with 50 and 100 μg/mL concentrations of ethyl acetate fraction (EAF) and butanol fraction (BF) and then incubated with 200 μM of $H_2O_2$ for 4 h. (B) Cell treated with various concentration of EAF. The values presented are the means ± SD (n =4).

with $H_2O_2$ only indicating that only the EAF protected HepG2 cells against $H_2O_2$-induced cytotoxicity (Fig. 2A). Pre-incubation of cells with EAF for 1 h inhibited the cell death induced by $H_2O_2$ in a dose-dependent manner (Fig. 2B).

**Effects of EAF on $H_2O_2$-induced cellular DNA damage.** Naturally, cells may recover from oxidative injury by repairing the damaged molecules to survive. However, serious damage, especially to DNA, may cause death via apoptosis or necrosis (Halliwell and Whiteman, 2004). The comet assay is a direct method of measuring DNA strand breaks, and detection is based on the formation of "DNA comets". Fig. 3A shows the comet tail formed following treatment with 200 μM $H_2O_2$ (positive control) at different concentration of EAF pre-treatment. The DNA damage significantly decreased in a dose-dependent manner when cells were treated with the EAF before exposure to $H_2O_2$ (Fig. 3B).

**Chemical characterization of EAF by LC-PDA-ESI-MS/MS.** A non-targeted LC-PDA-MS/MS chemical analysis of the EAF was performed to identify the components responsible for the protective effect against $H_2O_2$-induced toxicity in HepG2 cells. We performed UV spectrum at 310 nm and SIM analysis of major peaks of EAF with the corresponding mass value. Compounds were tentatively identified based on molecular ions of the parent, UV values, retention times, and elution order, as well as the fragmentation pattern described in the literature. The UV and MS data of the major compound, quercetin monoglycoside, were established on the basis of SIM analysis and by comparison with the published data (Rangkadilok et al., 2005). The results indicated the presence of a phenolic acid (gallic acid), phloridizin (Moon et al., 2011), three quercetin glycosides (Furlan et al., 2010), 2,6-dihydroxy-3,5-dimethyl-4-glucopyranosyl-benzophenone (Shu et al., 2010), guaijaverin (Hidetoshi and Danno, 2002), naringin, as well as other compounds (Table 2). In addition, literature survey revealed that most of these compounds exhibited antioxidant/ radical scavenging activities (Kroyer, 1986; Moon et al., 2001;

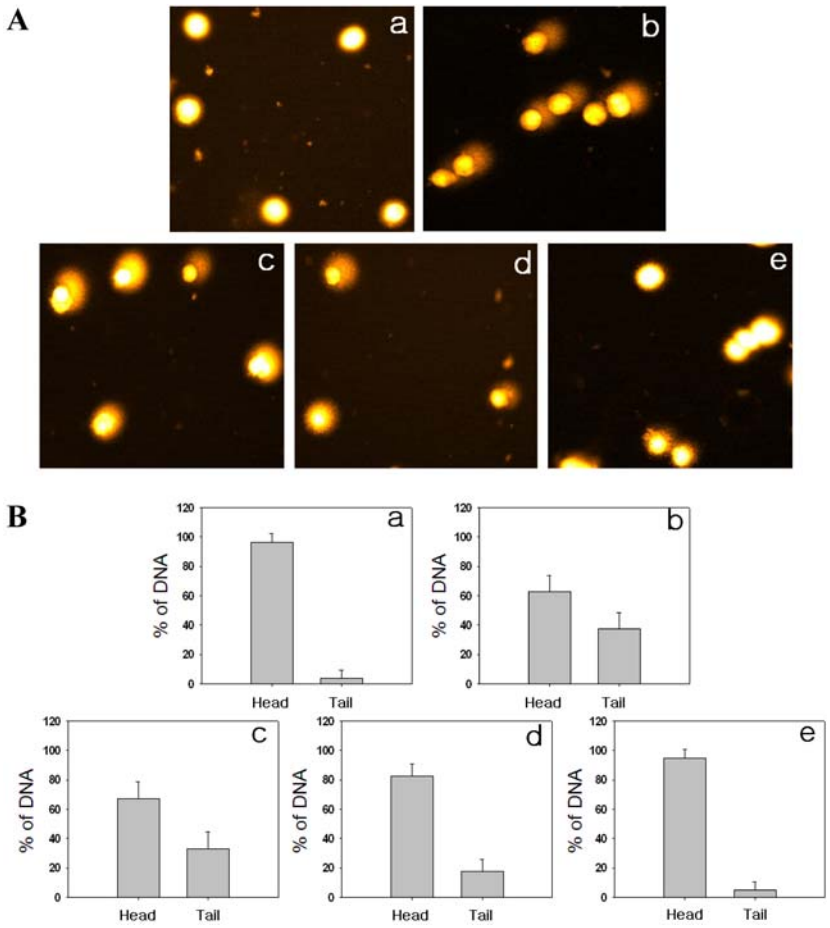


**Fig. 3** Protective effects of EAF on $H_2O_2$-induced DNA damage by comet assay. (A) HepG2 cells were pretreated with the 25–100 μg/mL of EAF for 1 h prior to the incubation with 200 μM of $H_2O_2$ for 4 h. (B) Graphical representation of comet assay. (a) Control. (b) 200 μM $H_2O_2$. (c) 25 μg/mL EAF+$H_2O_2$. (d) 50 μg/mL EAF+$H_2O_2$. (e) 100 μg/mL EAF+$H_2O_2$.

**Table 2** Chemical profile of the major peaks and identified compounds in the EtOAc fraction of *P. cattleianum* leaf

| Peak No | RT (min) | UV data | ESI $[M+H]^+$ | Fragmentation | Identified compound | % RPA |
|---|---|---|---|---|---|---|
| 1 | 1.42 | 224, 272 | 171.2 | 125.0 | Gallic acid | 2.9 |
| 2 | 5.79 | 218, 272 | 312.7 | 272.0, 155.2 | Unknown | 6.1 |
| 3 | 7.89 | 212, 287, 338 | 537.8 | 469.4, 312.7 | Unknown | 3.9 |
| 4 | 9.05 | 208, 275 | 437.3 | 275.3 | Phloridizin | 15.6 |
| 5 | 10.7 | 256, 353 | 479.1 | 303.2, 149.2 | Quercetin-3-glucuronide | 6.7 |
| 6 | 11.41 | 213, 265, 348 | 465.3 | 303.2, 149.1 | Quercetin monoglycoside (hexose) | 35.2 |
| 7 | 12.48 | 211, 276, 330 | 629.3 | 465.1, 303.1, 149.0 | Quercetin 3-diglycoside (hexoses) | 7.3 |
| 8 | 13.18 | 208, 271, 335 | 421.3 | 243.2, 149.2 | 2,6-dihydroxy-3,5-dimethyl-4-glucopyranosyl-benzophenone | 5.1 |
| 9 | 14.70 | 208, 276 | 500.2 | 439.2, 255.3 | Unknown | 3.5 |
| 10 | 15.15 | 208, 260, 353 | 435.2 | 303.1, 149.1 | Guaijaverin | 2.9 |
| 11 | 17.09 | 207, 261, 278 | 581.2 | 435.1, 273.2 | Naringin | 3.2 |
| 12 | 17.81 | 207, 289, 338 | 541.2 | 469.3 | Unknown | 3.6 |

[a]Retention time (min).
[b]Compounds tentatively identified based on parent molecular ions, UV values, retention times, and elution order, as well as the fragmentation pattern described in the literature.
[c]Relative peak area percentage (peak area relative to the total peak area %).

Yoshizumi et al., 2002; Ojewole, 2005; Wang, 2005). The percentage of relative peak area of the tentatively identified compounds was used to determine the relative concentrations of the constituents in the EAF of guava leaves. Overall, phloridizin (15.6%), quercetin-3-glucuronide (6.7%), quercetin monoglycoside (35.2%), and quercetin 3-diglycoside (7.3%), represent the major proportion (about 65%) of the whole fraction, and they might be responsible for the antioxidant activities.

Although effects of plant extracts and isolated compounds on DNA repair are still poorly studied, some findings show that polyphenols such as curcumin and quercetin increase DNA repair activity (Ramos et al., 2008). Several studies reported similar protective effect of plant product against DNA damage in human liver carcinoma HepG2 cells exposed to reactive species (Ramos et al., 2010). Previously, Musonda and Chipman (1998) also reported that quercetin inhibits hydrogen peroxide ($H_2O_2$)-induced NF-kappaB DNA binding activity and DNA damage in HepG2 cells. In addition, Tan et al. (2009) proposed that the effects of polyphenols could be due to three possible mechanisms: polyphenols scavenge $H_2O_2$, reducing the amount of DNA oxidative damage; polyphenols promote the effect of spontaneous enzymic repair; polyphenols rapidly repair the transient DNA damage resulting from $H_2O_2$ attack before enzymic repair initiation. Thus, the present results suggest that the EAF is rich in polyphenol compounds, and the biological effects of EAF were not, at least solely, caused by a single component, highlighting the potential advantage of using whole extracts rather than isolated compounds. Further studies on the chemical constituents of EAF responsible for the antioxidant activity, as well as the mechanism of their action by *in vivo* studies are under investigation in our lab.

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