

# Evaluation of Cellular Antioxidant and Antiproliferative Activities of Five Main *Phyllanthus Emblica* L. Cultivars in China

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The cell-based antioxidant activity assay as more biological relevant assay was considered to be more accurate to predict antioxidant activity *in vivo* than chemical activity assays. In the present study, the five main *Phyllanthus emblica* L. cultivars in China were subjected for cellular antioxidant activity based on HepG<sub>2</sub> cells as well as antiproliferative activity. Total phenolics, total flavonoids and oxygen radical absorbance capacity were also measured. The results showed that Qingyougan, Binggan and Boligan (832±100, 774±52 and 704±28 μmol of quercetin equivalents/100 g) had higher cellular antioxidant activity than Tianyougan and Yougan (553±50 and 457±24 μmol of quercetin equivalents/100 g) in phosphate buffered saline wash protocol whereas, Boligan (3735±217 μmol of quercetin equivalents/100 g) had the highest cellular antioxidant activity and Tianyougan (2025±171 μmol of quercetin equivalents/100 g) had the lowest cellular antioxidant activity in no phosphate buffered saline wash protocol. The highest and lowest antiproliferative activities were observed in Binggan and Tianyougan (median effective dose: 6.95±0.11 and 14.03±0.10 mg/ml), respectively. The significant correlation was only observed between total flavonoids and cellular antioxidant activity from no phosphate buffered saline wash protocol ( $R^2=0.908$ ,  $P<0.05$ ), and total flavonoids and antiproliferative activity ( $R^2=0.887$ ,  $P<0.05$ ), suggesting the major contribution of flavonoids to the bioactivities of *emblica*. Overall, the data obtained revealed that different *Phyllanthus emblica* L. cultivars had strong cellular antioxidant and antiproliferative activities, thus should be recommended to increase consumption for health.

**Key words:** *Phyllanthus emblica* L., cellular antioxidant activity, antiproliferative activity, oxygen radical absorbance capacity, phenolics, flavonoids

*Phyllanthus emblica* L., also known as Indian gooseberry, gooseberry, *Emblica officinalis*, emblica, *amla*, *amalki*, or *aonla*, is widely distributed in the tropical and subtropical areas of China, India, Indonesia and Thailand<sup>[1,2]</sup>. This plant has been widely used in many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Ayurveda medicine<sup>[3-5]</sup>. Various parts of the plant have been used to treat a range of ailments, but the most important part is the fruit. The fruit extract of *emblica* has been reported to possess anticancer<sup>[6]</sup>, antiinflammatory<sup>[7]</sup>, antiulcer<sup>[8]</sup>, antidiabetic<sup>[9]</sup>, cardioprotective<sup>[10]</sup>, and hepatoprotective activities<sup>[11]</sup> which, directly or indirectly attribute to its antioxidant activity since numerous diseases

or disorders are associated with free radicals and oxidative stress<sup>[12-14]</sup>.

Numerous studies have shown that the *emblica* fruit exhibits a high level of antioxidant capacity to inhibit lipid peroxidation<sup>[15,16]</sup> and scavenge free radicals<sup>[5,17-19]</sup> such as superoxide anion radical, hydrogen radical, hydrogen peroxide, nitric oxide radical, which

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may be due to high contents of phenolics, such as flavonoids (e.g. quercetin) and hydrolysable tannins (e.g. gallic acid and its derivatives, ellagic acid and its derivatives, isocorilagin, chebulanin, chebulagic acid, furosin, geraniin, mallotusin) [20-22]. Total phenolic content, as well as total flavonoid content, can vary among cultivars, which, may ultimately affect the overall antioxidant activity as well as other good therapeutic properties. Thus, it is of interest to explore the differences in phenolic content and antioxidant activity between different *emblica* cultivars so as to provide a more complete characterization on the *emblica*.

Previous studies on the antioxidant activity of *emblica* fruit have focused mainly on the chemical antioxidant activity assays, such as DPPH radical/ABTS radical/hydroxyl radical/superoxide anion radical scavenging assays [17,23], the ferric reducing/antioxidant power (FRAP) assay [23], and ferrous ion chelating ability assay [20]. However, the ability of the chemical antioxidant activity assay to predict *in vivo* activity is questioned [24] since biological systems are much more complex than the simple chemical mixtures employed. The cellular antioxidant activity (CAA) assay, a more biological representative method, has been developed to measure the antioxidant activity of antioxidants, dietary supplements, and foods in cell culture [25]. The CAA assay utilizes 2',7'-dichlorofluorescein diacetate (DCFH-DA) as a probe in cultured human HepG<sub>2</sub> cells, which is deacetylated by cellular esterases to form polar 2',7'-dichlorofluorescein (DCFH) and then fluoresces when oxidized by peroxy radicals to dichlorofluorescein (DCF). The HepG<sub>2</sub> cell-based CAAs of a variety of fruits and vegetables have been determined [26,27], but there is no report on the HepG<sub>2</sub> cell-based CAA of different kinds of *emblica*, despite that the CAA of *emblica* from Thailand was determined by using human myeloleukemic U937 cells, which showed that the water extract of *emblica* possess stronger cellular antioxidant effect than gallic acid [23].

The objective of this study was to measure the HepG<sub>2</sub> cell-based CAAs of the five main *Phyllanthus emblica* L. cultivars in China (Qingyougan, Yougan, Tianyougan, Boligan and Binggan), as well as the oxygen radical absorbance capacity (ORAC) and the antiproliferative activity on HepG<sub>2</sub> human liver cancer cell growth. Besides, total

phenolics and total flavonoids were also measured to further examine the correlations between antioxidant activity or antiproliferative activity and total phenolics or total flavonoids.

## MATERIALS AND METHODS

Folin-Ciocalteu reagent, gallic acid, catechin hydrate, fluorescein disodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH), 2',7'-dichlorofluorescein diacetate (DCFH-DA), 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (Hepes), Williams' Medium E (WME), trypan blue, dimethyl sulfoxide, and methylene blue were purchased from Sigma-Aldrich, St. Louis, MO, USA. Sodium hydroxide, sodium carbonate, aluminium chloride, sodium nitrite, acetone, ethanol, ethyl acetate, glutaraldehyde and acetic acid were obtained from Guangzhou Chemical, Guangzhou, China. Fetal bovine serum (FBS), 0.05% Trypsin-EDTA and Hanks' balanced salt solution (HBSS) were purchased from Gibco Life Technologies, Grand Island, NY, USA. The HepG<sub>2</sub> human liver cancer cells were obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA.

### Preparation of *Phyllanthus emblica* L. extracts:

The five main *Phyllanthus emblica* L. cultivars in China (Qingyougan, Yougan, Tianyougan, Boligan and Binggan), harvested at the edible maturity stage in Zhangpu County, Fujian Province between September and October, and authenticated by Fruit Tree Research Institute (Guangzhou, China), were purchased from a local market (Buji, Shenzhen, China). Extracts were prepared from the edible portions of fresh fruits using a modified method, as reported previously [26]. Briefly, in triplicate, a weight of 100 g of fresh *emblica* fruit was homogenized in 200 ml of 70% chilled acetone with a high speed homogenizer for 5 min. The mixture was then centrifuged at 12 000 g for 10 min. The supernatant was removed and the residue was again extracted with 200 ml of 70% chilled acetone. The centrifugation and the supernatant removal were also repeated again. The two aliquots of supernatant were pooled and evaporated at 45° to dryness. The final extracts were diluted with deionized water to a final volume of 10 ml and stored at -80° until use. Control extracts were prepared using the same extraction solvents and procedures without *emblica* fruits.

**Determination of total phenolic content:**

The total phenolic content was measured using the colorimetric Folin-Ciocalteu method reported previously<sup>[28]</sup> with slight modifications. Briefly, volumes of 0.4 ml of deionized water and 0.1 ml of diluted extracts were added to a test tube. A volume of 0.1 ml of Folin-Ciocalteu reagent was then added to the solution and allowed to react for 6 min. After reaction, volumes of 1 ml of 7% sodium carbonate and 0.8 ml of deionized water were added to the test tube. The color was developed for 90 min, and the absorbance was read at 760 nm using Spectra Max M2 spectrophotometer (MD, USA). The measurement was compared to a standard curve of gallic acid, obtained from a known concentration range of gallic acid standards prepared similarly as the sample. All results were expressed as grams of gallic acid equivalents (GAE) per 100 g fresh *emblica* fruit. Data were shown as mean±SD for three replications.

**Determination of total flavonoid content:**

The total flavonoid content was measured using aluminium chloride-sodium nitrite method<sup>[29]</sup> with some modifications in this study. That is, volumes of 2 ml of diluted extracts and 75 µl of 5% sodium nitrite was added to the test tube and allowed to react for 6 min. Then, a 150 µl aliquot of 10% aluminium chloride was added to the test tube. After 5 min, a 0.5 ml aliquot of 1 M sodium hydroxide was added, and the mixture was diluted to a final volume of 3 ml with deionized water. The absorbance of the final mixture was read immediately at 510 nm using Spectra Max M2 spectrophotometer (MD, USA). The measurement was compared to the standard curve of catechin and expressed as milligrams of catechin equivalents per 100 g fresh *emblica* fruit. Data were shown as mean±SD for three replications.

**Determination of total antioxidant activity:**

The total antioxidant activity of the five *emblica* cultivars extracts was measured using the oxygen radical absorbance capacity (ORAC) assay<sup>[26]</sup>. That is, a 20 µl aliquot of blank, Trolox standard, or *emblica* extracts in 75 mM potassium phosphate buffer (pH 7.4, working buffer), was added to triplicate wells in a black, clear-bottom, 96-well microplate. The triplicate samples were distributed throughout the microplate and were not placed side-by-side, to avoid any effects on readings due to location. A volume of 200 µl of 0.96 µM fluorescein in working buffer was added to each well and incubated at 37° for 20 min,

with intermittent shaking, before the addition of 20 µl of freshly prepared 119 mM AAPH in working buffer. The microplate was immediately inserted into a Fluoroskan Ascent FL plate reader (MD) at 37°. The decay of fluorescence at 538 nm was measured with excitation at 485 nm every 4.5 min for 2.5 h. The areas under the fluorescence versus time curve for the samples minus the area under the curve for the blank were calculated and compared to a standard curve of the areas under the curve for 6.25, 12.5, 25, and 50 µM Trolox standards minus the area under the curve for blank. ORAC values were expressed as micromoles of Trolox equivalents (TE) per 100 g fresh *emblica* fruit. Data were shown as mean±SD for three replications from one experiment.

**Cell culture:**

HepG<sub>2</sub> cells were grown in growth medium (WME supplemented with 5% FBS, 10 mM Hepes, 5 µg/ml insulin, 0.05 µg/ml hydrocortisone, 50 units/ml penicillin, 50 µg/ml streptomycin and 100 µg/ml gentamicin) and were maintained at 37° in 5% CO<sub>2</sub> as described previously<sup>[26]</sup>. Cells used in this study were between passages 12 and 35.

**Cytotoxicity:**

The cytotoxicity of the five *emblica* cultivars extracts toward HepG<sub>2</sub> cells was measured using the colorimetric methylene blue assay reported previously<sup>[25]</sup>. HepG<sub>2</sub> cells were seeded at 4×10<sup>4</sup>/well on a 96-well microplate in 100 µl of growth medium at 37°. Twenty-four hours after seeding, the growth medium was removed, and the cells were washed with 100 µl of phosphate buffered saline (PBS). Then, a volume of 100 µl of treatment medium (WME supplemented with 2 mM L-glutamine and 10 mM Hepes) containing various concentrations (10, 25, 50, 100, 150, 200 and 250 mg/ml) of extracts were applied to the cells, and the microplates were incubated at 37° for 24 h. The treatment medium was removed, and the cells were washed with PBS. A volume of 50 µl/well methylene blue staining solution (98% HBSS, 0.67% glutaraldehyde, 0.6% methylene blue) was applied to each well, and the microplate was incubated at 37° for 1 h. The dye was removed, and the plate was immersed in fresh deionized water until the water was clear. The water was tapped out of the wells, and the microplate was allowed to air-dry briefly before 100 µl of elution solution (49% PBS, 50% ethanol, 1% acetic acid) was added to each well. Then the microplate was

placed on a bench-top shaker for 20 min to allow uniform elution. The absorbance was read at 570 nm with blank subtraction using the Spectra Max M2 spectrophotometer (MD, USA). Concentrations of extracts that decreased the absorbance to a significant difference ( $P < 0.05$ ) when compared with the control were considered to be cytotoxic.

#### Cellular antioxidant activity of *emblica* extracts:

The CAA assay was reported previously<sup>[25-27]</sup>. Briefly, HepG<sub>2</sub> cells were seeded at a density of  $6 \times 10^4$ /well on a 96-well microplate in 100  $\mu$ l of growth medium/well. Twenty four hours after seeding, the growth medium was removed, and the wells were washed with 100  $\mu$ l of PBS. Wells were treated in triplicate for 1 h with 100  $\mu$ l of treatment medium containing solvent control, control extracts or extracts plus 25  $\mu$ M DCFH-DA. When a PBS wash was utilized, wells were washed with 100  $\mu$ l of PBS. Then 600  $\mu$ M AAPH was added to the cells in 100  $\mu$ l of HBSS, and the 96-well microplate was placed into a Fluoroskan Ascent FL plate reader at 37°. Emission at 538 nm was measured after excitation at 485 nm every 5 min for 1 h.

#### Quantification of CAA:

As described previously<sup>[25]</sup>, the area under fluorescence versus time curve was integrated after blank subtraction and subtraction of the initial fluorescence values. The CAA values of extracts at each concentration were calculated using the following formula,  $CAA \text{ unit} = 1 - \frac{\int SA}{\int CA}$ , where,  $\int SA$  is the integrated area under the sample fluorescence versus time curve and  $\int CA$  is the integrated area from the control curve. The median effective dose ( $EC_{50}$ ) was determined from the median effect plot of  $\log(f_a/f_u)$  versus  $\log(\text{dose})$ , where  $f_a$  is the fraction affected (CAA unit) and  $f_u$  is the fraction unaffected (1-CAA unit) by the treatment. The  $EC_{50}$  values were reported as mean $\pm$ SD for triplicate sets of data obtained from the same experiment.  $EC_{50}$  values were converted to CAA values, expressed as micromoles of quercetin equivalents (QE) per 100 g fresh *emblica* fruit, using the mean  $EC_{50}$  value for quercetin from five separate experiments.

#### Measurement of antiproliferative activity:

The antiproliferative activity of the five *emblica* cultivars extracts was determined by measurement of the inhibition of HepG<sub>2</sub> human liver cancer cell

proliferation using the colorimetric methylene blue assay reported previously<sup>[30]</sup>. Briefly, HepG<sub>2</sub> cells were seeded at a density of  $2.5 \times 10^4$ /well on a 96-well microplate in 100  $\mu$ l of growth medium/well. Four hours after seeding, the growth medium was removed, and media containing various concentrations (0, 5, 10, 25, 50, 100, 150 and 200 mg/ml) of extracts were added to the wells. Control cultures received the extraction solution minus the extracts, and blank wells contained 100  $\mu$ l of growth medium without cells. After 96 h of incubation, the treatment medium was removed, the cells were washed with PBS, dyed, washed and eluted. The absorbance was read at 570 nm with blank subtraction. Cell proliferation (percent) was calculated from the comparison between the sample absorbance reading at each concentration and the control absorbance, using at least three replications for each sample. The median effective dose ( $ED_{50}$ ) was determined and expressed as mg of extract per ml. Data were shown as mean $\pm$ SD.

#### Statistical analysis:

All data were reported as mean $\pm$ SD for three replications. Statistical analysis was performed using SPSS V20.0 software (SPSS Inc, Chicago). Differences between means were analyzed by One-Way ANOVA test. Correlations between various parameters were also investigated using regression analysis. Significance was determined at  $P < 0.05$ .

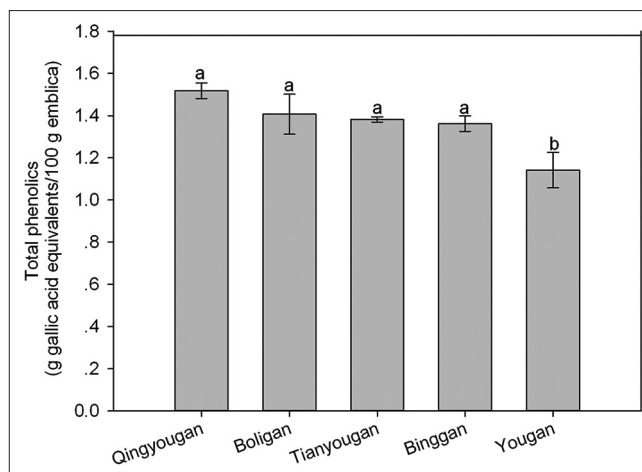
## RESULTS AND DISCUSSION

The total phenolic content of the five main *Phyllanthus emblica* L. cultivars in China was determined from their 70% acetone extracts by using the Folin-Ciocalteu method (fig. 1). Seventy percent acetone was used as extraction solvent in this study based on our preliminary experiments, which, showed that the *emblica* extract from 70% acetone had higher content of phenolics and higher level of antioxidant activity than the extract from other solvents. Singh *et al.*<sup>[31]</sup>, also reported that the acetone extract exhibited better hydroxyl radical scavenging activity than methanol or chloroform extract for the *emblica* containing *triphala*. It could be found from fig. 1 that Yougan had the lower phenolic content ( $1.142 \pm 0.084$  g of GAE/100 g) as being compared with other four *emblica* cultivars extracts ( $P < 0.05$ ), and there was no significant difference in the total phenolic contents between Qingyougan ( $1.518 \pm 0.037$  g of GAE/100 g),

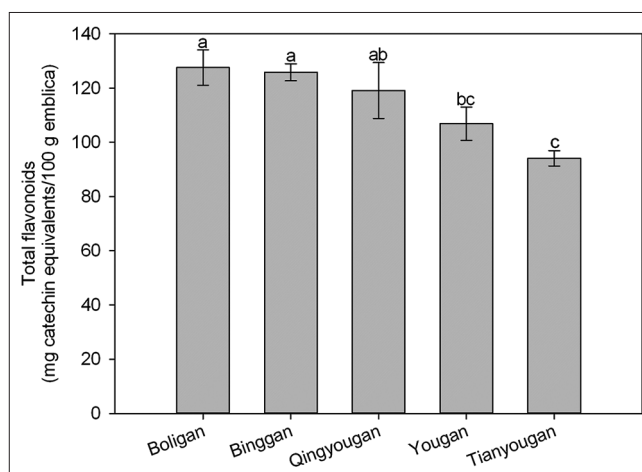
Boligan ( $1.407 \pm 0.095$  g of GAE/100 g), Tianyougan ( $1.382 \pm 0.013$  g of GAE/100 g) and Binggan ( $1.362 \pm 0.037$  g of GAE/100 g), averaging 1.417 g of GAE/100 g. There was a 75% difference in phenolic content between the highest and the lowest cultivars ( $P < 0.05$ ). It should be noted that the phenolic content determined in this study was lower than the previous reports<sup>[18,32]</sup>. The main reason lied in that data expressed here were based on the 100 g fresh fruit rather than 100 g dried fruit.

Flavonoid contents of the five *Phyllanthus emblica* L. cultivars are shown in fig. 2. Boligan, Binggan and Qingyougan had the highest total flavonoid content ( $127.6 \pm 6.5$ ,  $125.8 \pm 3.1$ , and  $117.8 \pm 10.3$  mg of catechin equivalents/100 g, respectively), followed by Yougan ( $106.7 \pm 6.1$  mg of catechin equivalents/100 g), which was statistically similar to Qingyougan. The lowest flavonoid content was observed in Tianyougan ( $94.1 \pm 2.9$  mg of catechin equivalents/100 g), but statistically, this cultivar was similar to Yougan. There was a 74% difference in flavonoid content between the highest and the lowest cultivars ( $P < 0.05$ ), similar to phenolic content, despite of the different relative ranking of cultivars for phenolics and flavonoids.

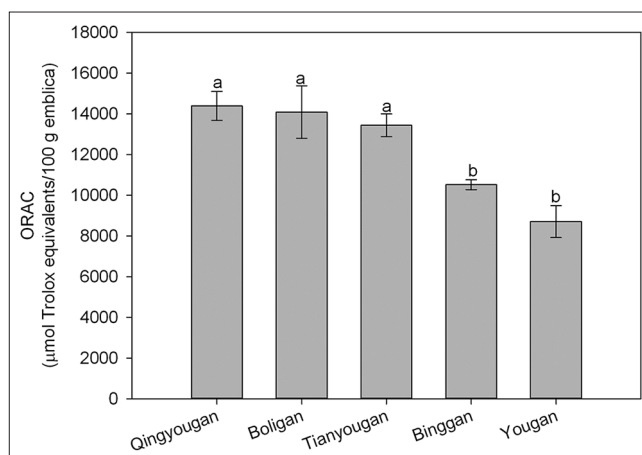
The total antioxidant activities of the five *Phyllanthus emblica* L. cultivars were measured by the ORAC assay and expressed as micromoles of Trolox equivalents (TE) per 100 g of *emblica* (fig. 3). Qingyougan ( $14387.0 \pm 707.2$   $\mu\text{mol}$  of TE/100 g), Boligan ( $14087.5 \pm 1289.5$   $\mu\text{mol}$  of TE/100 g) and Tianyougan ( $13433.6 \pm 558.6$   $\mu\text{mol}$  of TE/100 g) ( $P > 0.05$ ) which, were not significantly different from each other ( $P > 0.05$ ), exhibited higher peroxy radical scavenging ability than Binggan and Yougan ( $10519.2 \pm 237.2$ ,  $8708.1 \pm 784.7$   $\mu\text{mol}$  of TE/100 g, respectively), which were also statistically similar to each other ( $P > 0.05$ ). There was a 60% difference in antioxidant activity between the highest and the lowest cultivars ( $P < 0.05$ ). Besides, the relative ranking of cultivars for ORAC values was the same as that for total phenolic content. Additionally, a significantly positive relationship was observed between total phenolics and ORAC values ( $R^2 = 0.891$ ,  $P < 0.05$ ), indicating the major contribution of the phenolics to the peroxy radical scavenging ability. The positive correlation between phenolics and antioxidant activity was also reported in many other fruits and vegetables<sup>[26,27]</sup>.



**Fig. 1: Total phenolic content of the five *emblica* cultivars.** All data were shown as mean  $\pm$  SD from triplicate experiments. Bars with no letters in common are significantly different ( $P < 0.05$ ).



**Fig. 2: Total flavonoid content of the five *emblica* cultivars.** All data were shown as mean  $\pm$  SD from triplicate experiments. Bars with no letters in common are significantly different ( $P < 0.05$ ).



**Fig. 3: Oxygen radical absorbance capacity (ORAC) of the five *emblica* cultivars.** All data were shown as mean  $\pm$  SD from triplicate experiments. Bars with no letters in common are significantly different ( $P < 0.05$ ).

Since the consideration of some aspects of cell uptake, metabolism, and distribution of bioactive compounds, the CAA assay is an improvement over the traditional chemical antioxidant activity assays<sup>[25]</sup>. Thus, the cellular antioxidant activities of the five *Phyllanthus emblica* L. cultivars were evaluated using the CAA assay. The EC<sub>50</sub> for the five cultivars, along with their median cytotoxicity doses (CC<sub>50</sub>), is listed in Table 1. The CAA was measured using two protocols, as described previously<sup>[25-27]</sup> in the PBS wash, the HepG<sub>2</sub> cells were washed with PBS between the *emblica* extract and AAPH treatments; in the no PBS wash, the HepG<sub>2</sub> cells were not washed between the *emblica* extract and AAPH treatments.

The CAA values of the five *Phyllanthus emblica* L. cultivars in the PBS wash protocol are shown in fig. 4a. Qingyougan (832±100 µmol of QE/100 g), Binggan (774±52 µmol of QE/100 g) and Boligan (704±28 µmol of QE/100 g), which were not significantly different from each other ( $P>0.05$ ), had the greater cellular antioxidant activity than Tianyougan (553±50 µmol of QE/100 g) and Yougan (457±24 µmol of QE/100 g), which were also not significantly different from each other ( $P>0.05$ ). There was significant difference between Boligan and Tianyougan ( $P<0.05$ ). There was a 59% difference in CAA values from the PBS wash protocol between the highest and the lowest cultivars ( $P<0.05$ ), similar to the ORAC values. Additionally, for ORAC values and CAA values from the PBS wash, the highest and the lowest cultivars were identical, that is, Qingyougan of the highest while Yougan of the lowest.

The CAA values of the five *Phyllanthus emblica* L. cultivars in the no PBS wash protocol are shown in fig. 4b. Boligan showed the highest cellular antioxidant activity with a CAA value of 3735±217 µmol of QE/100 g, followed by Qingyougan (3164±426 µmol of QE/100 g), Binggan (2918±240 µmol of QE/100 g), Yougan (2373±85 µmol of QE/100 g) and Tianyougan (2025±171 µmol of QE/100 g). There were no significant differences between Boligan and Qingyougan, Qingyougan and Binggan, Binggan and Yougan, Yougan and Tianyougan ( $P>0.05$ ). There was a 54% difference in CAA values from the no PBS wash protocol between the highest and the lowest cultivars ( $P<0.05$ ). Similar to the CAA values from the no PBS wash protocol, the highest and the lowest flavonoid contents were also observed in Boligan and Tianyougan, respectively. Besides, correlation analysis also showed that total flavonoids were significantly related to CAA values from the no PBS wash protocol ( $R^2=0.908$ ,  $P<0.05$ ), suggesting

**TABLE 1: CELLULAR ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF THE FIVE PHYLLANTHUS EMBLICA L. CULTIVARS**

Cultivars	EC <sub>50</sub> (mg/ml)		Cytotoxicity (mg/ml)
	PBS wash	no PBS wash	
Qingyougan	1.11±0.13	0.278±0.040	>20
Binggan	1.12±0.07	0.271±0.023	>20
Boligan	1.24±0.05	0.212±0.013	>20
Tianyougan	1.67±0.16	0.393±0.032	>20
Yougan	2.01±0.10	0.367±0.013	>20

EC<sub>50</sub> is the median effective dose, and the data were shown as mean±SD from triplicate experiments. PBS: phosphate buffered saline

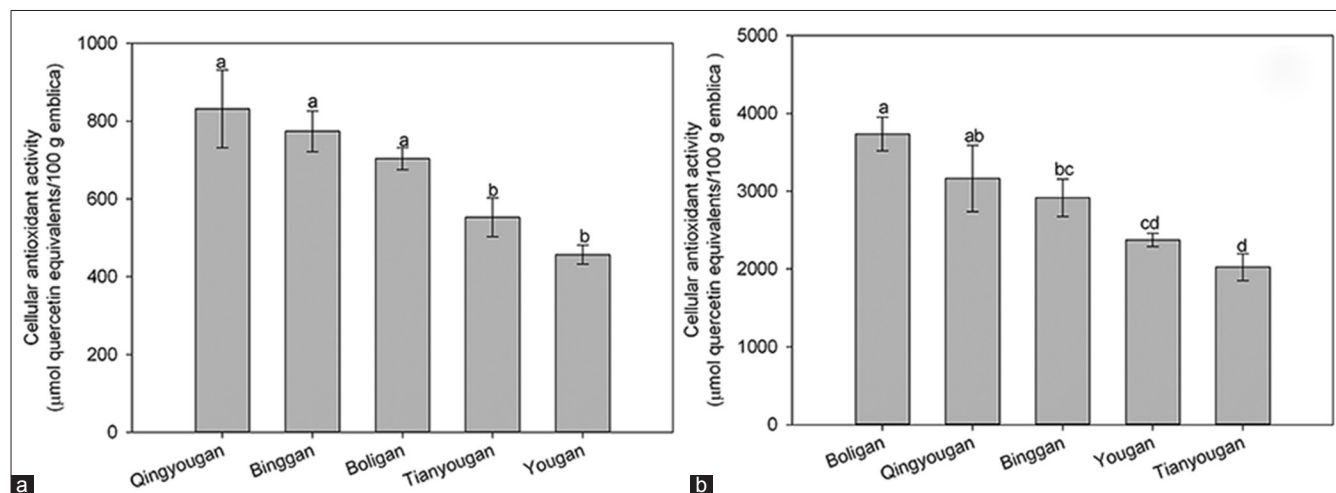


Fig. 4: Cellular antioxidant activity (CAA) of the five *emblica* cultivars.

a and b showed CAA in the PBS wash protocol and no PBS wash protocol, respectively. All data were shown as mean±SD from triplicate experiments. Bars with no letters in common are significantly different ( $P<0.05$ ).

that flavonoids might be responsible for the cellular antioxidant activity of the *emblica*. Quercetin, the major flavonoid in *emblica*<sup>[32]</sup>, was reported to possess strong cellular antioxidant activity<sup>[25]</sup>.

In addition, all the five *emblica* cultivars showed much higher level of CAAs than previously reported vegetables and fruits<sup>[26,27]</sup>, consistent with the ORAC values, which might be attributed to much higher phenolic content in *emblica*. However, no significant correlation was observed in the five *emblica* cultivars between total phenolics and CAA values from the PBS wash protocol ( $R^2=0.835$ ,  $P>0.05$ ) and no PBS wash protocol ( $R^2=0.483$ ,  $P>0.05$ ), which, might be due to the synergistic or antagonistic interactions between different phenolics<sup>[33]</sup>. Besides, all the five *emblica* cultivars extracts displayed dramatically lower CAA values when a PBS wash was done between the *emblica* extract and AAPH treatments, implying that the some bioactive compounds in the tested extract adsorbed loosely to the membrane and were taken up less readily. The similar results were shown in gallic acid and ascorbic acid<sup>[25]</sup>, which, were of large amount in *emblica*<sup>[22,34]</sup>.

The antiproliferative activities of the five *Phyllanthus emblica* L. cultivars were determined from the inhibition of their extracts toward the growth of HepG<sub>2</sub> cells *in vitro* using the methylene blue assay. In fig. 5, the proliferation of HepG<sub>2</sub> cell was inhibited in a dose-dependent manner after exposure to all five extracts. The antiproliferative activity

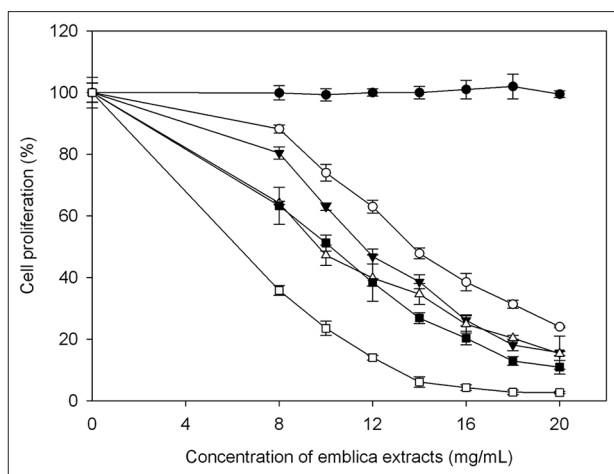


Fig. 5: Percent inhibition of HepG<sub>2</sub> cell proliferation by the five *emblica* cultivars extracts.

All data were shown as mean $\pm$ SD from triplicate experiments.  
 —●— Control, —○— Tianyougan, —▼— Yougan, —△— Boligan,  
 —■— Qingyougan, —□— Binngan.

was also expressed as the median effective dose, ED<sub>50</sub> (fig. 6) where, a lower ED<sub>50</sub> value signified a higher antiproliferative activity. Binggan had the greatest antiproliferative activity with the lowest ED<sub>50</sub> value (6.95 $\pm$ 0.11 mg/ml), followed by Qingyougan and Boligan (9.95 $\pm$ 0.30 and 10.06 $\pm$ 0.49 mg/ml, respectively), and then Yougan (11.83 $\pm$ 0.12 mg/ml). The ED<sub>50</sub> of Tianyougan (14.03 $\pm$ 0.10 mg/ml) was significantly higher than all other cultivars ( $P<0.05$ ), in other words, Tianyougan was of the lowest antiproliferative activity. Correlation analysis showed that the antiproliferative activity was only significantly correlated to the total flavonoid content ( $R^2=0.887$ ,  $P<0.05$ ), suggesting the contribution of flavonoid to the antiproliferative activity. Liu *et al.*<sup>[2]</sup> reported that quercetin, kaempferol and their glycosides exhibited inhibitory effect against MCF-7 cells. Acylated apigenin glucoside, a flavonoid compound from *emblica*, was also reported to possess potent inhibitory on the growth of tumor cell lines<sup>[35]</sup>.

In this study, the antioxidant and antiproliferative activities of the five main *Phyllanthus emblica* L. cultivars in China (Qingyougan, Yougan, Tianyougan, Boligan and Binggan), as well as total phenolics and flavonoids, were evaluated from their 70% acetone extracts. The results indicated that total phenolics were significantly correlated to ORAC values ( $R^2=0.891$ ,  $P<0.05$ ), while total flavonoids were significantly correlated to CAA values from no PBS wash protocol ( $R^2=0.908$ ,  $P<0.05$ ) and antiproliferative activity ( $R^2=0.887$ ,

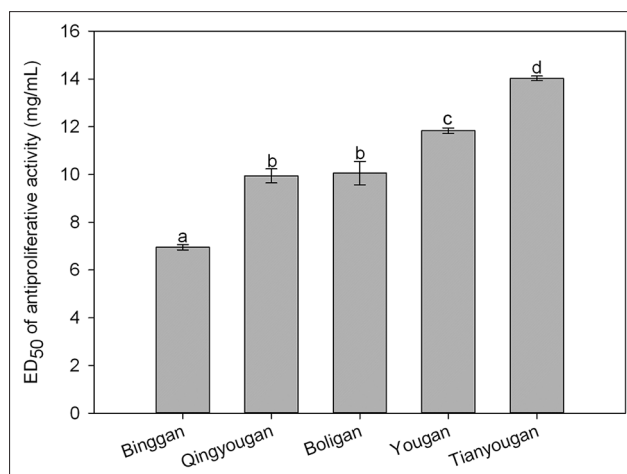


Fig. 6: ED<sub>50</sub> of antiproliferative activity by extracts of the five *emblica* cultivars.

ED<sub>50</sub> is the median effective dose, and the data were shown as mean $\pm$ SD from triplicate experiments. Bars with no letters in common are significantly different ( $p<0.05$ ).

$P < 0.05$ ). Although the CAA values, ORAC values and antiproliferative activities were not significantly correlated with each other for the five *emblica* cultivars in this study, the significant differences were observed among cultivars in respect to the above activities as well as the total phenolics and flavonoids. This knowledge could be useful for consumers to estimate the value of each cultivar. However, a more detailed investigation on the individual phenolic compound present in different *emblica* cultivars needs to be carried out so as to provide a more complete characterization on the health benefits of antioxidant and antiproliferative activities of different *emblica* cultivars. Overall, this study shows that different *Phyllanthus emblica* L. cultivars are of strong antioxidant and antiproliferative activities, thus should be recommended to eat daily for health.

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#### Conflict of interest:

There are no conflicts of interest.

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