

## Bioactivity and Stability Studies of Anthocyanin-Containing Extracts from *Garcinia mangostana* L. and *Etlingera elatior* Jack

(Aktiviti Biologi dan Kajian Kestabilan Ekstrak mengandungi Antosianin daripada *Garcinia mangostana* L. dan *Etlingera elatior* Jack)

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### ABSTRACT

*Anthocyanin-containing extract (ACE) were prepared from the pericarp of Garcinia mangostana L. (mangosteen) and the inflorescence part of Etlingera elatior Jack (torch ginger) by using acidified methanol as extraction solvent. Our present study focuses on the antioxidant and cytotoxic activity and the effect of temperature, light and pH on stability of ACE derived from G. mangostana and E. elatior. The monomeric anthocyanin and total phenolic content in E. elatior was higher (43.42±0.01 mg/L dan 10.07±0.01 gGAE/100 g) compared to G. mangostana (15.03±0.01 mg/L and 3.29±0.01 gGAE/100 g). Both ACE of E. elatior and G. mangostana exhibited free radical scavenger activity, with IC<sub>50</sub> value of 14.90±1.02 mg/mL and 15.50±0.52 mg/mL, respectively, in 2,2'-diphenyl-1-picrylhydrazyl assay. ACE of E. elatior was also found to be a good reducing and possessed higher absorbance values at concentrations range from 1.25 to 20 mg/mL in FRAP assay. ACE of E. elatior exhibited mild cytotoxicity on human ovarian SKOV-3 cell line with IC<sub>50</sub> values of 54.32 ± 4.60 µg/mL. The effects of light and temperature on ACE stability were performed in different environmental conditions, which promote the destabilization of anthocyanin molecules. ACE stability of G. mangostana were less resist to the effect of light but very susceptible to the prolonged effect of heat after 2 h exposure compared to the ACE in E. elatior. The different in pH highly influence the stability of both ACE which can be observed from the decrease in absorbance readings.*

*Keywords: Antioxidant; anthocyanin; cytotoxic; Etlingera elatior Jack; Garcinia mangostana L.*

### ABSTRAK

*Antosianin yang mengandungi ekstrak (ACE) telah disediakan daripada perikarp Garcinia mangostana L. (Manggis) dan bahagian bunga Etlingera elatior Jack (Kantan) dengan menggunakan metanol berasid sebagai pelarut pengekstrakan. Kajian ini memberi tumpuan kepada aktiviti antioksidan dan sitotoksik dan kesan suhu, cahaya dan pH pada kestabilan ACE daripada G. mangostana dan E. elatior. Kandungan antosianin monomer dan jumlah kandungan fenolik dalam E. elatior adalah lebih tinggi (43.42±0.01 mg/L dan 10.07±0.01 gGAE/100 g) berbanding dengan G. mangostana (15.03±0.01 mg/L dan 3.29±0.01 gGAE/100 g). Kedua-dua ACE E. elatior dan G. mangostana menunjukkan aktiviti pemakan radikal bebas dengan nilai IC<sub>50</sub> sebanyak 14.90±1.02 mg/mL dan 15.50±0.52 mg/mL, masing-masing dalam asei 2,2'-difenil-1-picrylhydrazyl. ACE E. elatior juga didapati mempunyai penurunan yang baik dan nilai absorbans yang tinggi pada kepekatan antara 1.25 hingga 20 mg/L dalam asei FRAP. ACE E. elatior menunjukkan sitotoksiti sederhana pada leluhur sel kanser SKOV-3 ovari dengan nilai IC<sub>50</sub> 54.32 ± 4.60 µg/mL. Kesan cahaya dan suhu terhadap kestabilan ACE telah dijalankan dalam keadaan persekitaran yang berbeza, yang menggalakkan ketidakstabilan molekul antosianin. Kestabilan ACE G. mangostana kurang menahan kesan cahaya tetapi sangat terdedah kepada kesan cahaya yang berpanjangan selama dua jam berbanding ACE E. elatior. Perbezaan dalam pH sangat mempengaruhi kestabilan kedua-dua ACE yang dapat dilihat daripada penurunan dalam bacaan absorbans.*

*Kata kunci: Antioksidan; antosianin; Etlingera elatior Jack; Garcinia mangostana L.; sitotoksik*

### INTRODUCTION

Food colorants have the ability to stimulate our moods and appetite towards food through the appearances of the colour. The first impression of the food is not only based on their physical appearance, but is also comes with the judgement whether the colorant used is safe and whether it provide any health benefit or not. Increasingly, food

producers are turning to natural-based food colorants as the synthetic colorant had been claimed to possess negative side effect and could potentially be toxic for human. The natural-based food colorants are usually extracted from the plant material and most of them are anthocyanin. The increase in demand on the natural colorant as compared to the synthetic colorant is due to many aspects involving

health and hygiene, pharmaceutical activities, nutrition, and also environmental consciousness (Chattopadhyay et al. 2008).

Anthocyanin are water soluble flavonoid pigments found in varieties of flowers, fruits and vegetables that, generating the characteristic reddish, bluish and purple hues (Jaakola 2013). Anthocyanin have raised a growing interest for natural colorants not only due to their extensive range of colours but also its enormous beneficial health effects reported, such as anti-inflammatory (Cassidy et al. 2015; Luo et al. 2014), anti-microbial (Junqueira-Goncalves et al. 2015), anti-carcinogenic (Rugina et al. 2012) and anti-diabetes (Sancho & Pastore 2012). Existing evidence indicates that anthocyanin is not only promoting positive therapeutic properties, but they also non-toxic and non-mutagenic. However, the anthocyanin are extremely unstable, easily degraded in the isolated form and the stability of anthocyanin is greatly affected by factors such as pH, temperature, oxygen and light (Reque et al. 2014).

Therefore, this project aims to study the biological potential of the anthocyanin-containing extracts (ACE) from two selected Malaysian plants, *Etilingera elatior* Jack and *Garcinia mangostana* L. The present study also aimed to investigate the stability of the ACE from both samples stored alone as a function of time, temperature, and light, and when incubated in biologically relevant buffers in different pH, for their potential application as a natural food colorant.

## MATERIALS AND METHODS

### RAW MATERIALS

Fruits of *Garcinia mangostana* L. (Mangosteen) and the inflorescences part of *Etilingera elatior* Jack (Torch ginger) were purchased from the local market around Petaling Jaya in December 2013. The pericarp of *G. mangostana* was separated manually removing any hint of flesh and the inflorescences part of *E. elatior* was removed and it was subsequently cut into small pieces. Both samples were cleaned with distilled water to remove any extraneous materials and dried at room temperature before being stored frozen at -20°C until processed for these present studies.

### PREPARATION OF ANTHOCYANIN-CONTAINING EXTRACTS (ACE)

The extraction of anthocyanin-containing extracts (ACE) was accomplished according to Longo and Vasapollo (2006). Both the pericarp and inflorescences part were separately crushed with mortar and pestle until they become powdery form. ACE was extracted from powder of *G. mangostana* and *E. elatior* with 0.1% HCl (v/v) in methanol in 20 h at room temperature, in darkness. The mixture was filtered and the filtrate was dried using rotary evaporator (Buchi, Switzerland) at 40°C and dried ACE was obtained for further study.

### MONOMERIC ANTHOCYANIN CONTENT

Monomeric anthocyanin content was determined using the pH-differential method of Giusti and Wrolstad (2001). ACE of *G. mangostana* and *E. elatior* were dissolved in potassium chloride buffer (KCl, 0.025 M, pH1.0) and sodium acetate buffer (CH<sub>3</sub>COONa·3H<sub>2</sub>O, 0.4 M, pH4.5) with a pre-determined dilution factor and was then allowed to reach equilibrium in 15 min. The absorbance of each measured samples were read at 510 and at 700 nm (haze correction). The absorbance (A) of the diluted sample was then calculated as follows:

$$A = (A_{510} - A_{700})_{\text{pH1.0}} - (A_{510} - A_{700})_{\text{pH4.5}}$$

The monomeric anthocyanin pigment concentration in the original sample was calculated using following formula:

$$\text{Monomeric anthocyanin content (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l}$$

where, MW is the molecular weight of main anthocyanin (cyanidin-3-glucoside MW = 449.2); DF is the dilution factor; and the molar absorptivity constant of main anthocyanin (cyanidin-3-glucoside  $\epsilon$  = 26,900) were used.

### TOTAL PHENOLIC CONTENT (TPC)

The phenolic content in ACE of *G. mangostana* and *E. elatior* was determined by Folin-Ciocalteu method (Ge & Ma 2013) at 765 nm and the quantification was done on the basis of the standard curve of gallic acid concentration ranging between 0 and 2 mg/mL ( $r^2 = 0.9598$ ). The amount of the TPC was calculated by using the following formula:

$$T (\text{GAE}) = C,$$

where T (GAE) is the total phenolic content that is express as g gallic acid equivalent (GAE) per 100 g; C is the concentration of gallic acid determined from the standard curve (mg/mL); V is the volume used during the assay (mL); and M is the mass of extract used during the assay (g).

### STABILITY STUDIES OF ACE FROM *G. MANGOSTANA* AND *E. ELATIOR* AT VARIOUS PARAMETERS

Stability studies were carried out according to the methods proposed by Reshmi et al. (2012) with slight modifications. The ACE of *G. mangostana* and *E. elatior* were prepared in acidified methanol.

**Temperature** The effects of temperature on ACE stability was done with samples inside capped glass vials covered with aluminium foil, sealed with parafilm and immersed in a water bath at 99°C ( $\pm 1^\circ\text{C}$ ) for 0, 30, 60, 90 and 120 min. The absorbance was recorded after each period of time at wavelength 540 nm.

**Light** Light effect on ACE stability was performed in two conditions, which are with samples inside capped glass vials covered with aluminium foil, sealed with parafilm and exposed to a 23 W white fluorescent light at a distance of 26 cm and in the dark. The absorbance was recorded at 520 nm for 0, 1, 3, 5 and 7 day's duration. The results for both stability studies were expressed as ACE survival rate calculated as follows:

$$\text{Survival rate (\%)} = A / A_0 \times 100,$$

where  $A$  is the absorbance of ACE after a period of time; and  $A_0$  is the absorbance of the initial ACE.

**pH** 0.05 M (2 µg/mL) of ACE of the samples was dissolved in acidified methanol inside capped glass vials. Each sample solution was then divided into five equal portions, dried, and dissolved in appropriate volumes of buffer. The glass vials were wrapped with aluminium foil and sealed with parafilm. The solvents used for preparations of the buffer-solutions were: (A) 0.2 M KCl, (B) 0.2 M HCl, (C) 0.1 M  $\text{KHC}_8\text{O}_4\text{H}_4$ , (D) 0.1 M HCl, and (E) 0.1 M NaOH.

The pH-values were measured and the absorbance of each ACE solution was recorded at 520 nm using spectrophotometer (Thermo Fisher Scientific, US). Each absorbance was recorded on day 0, 1, 2, 3, 4, 5, 6 and 7. The samples were kept in a refrigerator (25°C) between the measurements.

#### BIOACTIVITY STUDIES

**Neutral Red Uptakes (NRU) Cytotoxicity Assay** The cytotoxicity potential of ACE of *G. mangostana* and *E. elatior* was performed according to the methods described by Ramasamy et al. (2012). Human ovarian SKOV-3 cancer cells were detached from the flask with 1 mL solution of acutase in phosphate buffered saline solution (PBS) pH7.4. The cell pellet was obtained by centrifugation at 1000 rpm for 5 min. Cells were then seeded in 96-well micro titer plate at a concentration of 30000 cell/mL and then incubated in a  $\text{CO}_2$  incubator at 37°C for 3 h to allow the cells to adhere before addition of the test agents. After 3 h, the ACE were then added to the wells at concentrations of 1, 10, 25, 50, 75 and 100 µg/mL and the plates were further incubated for 72 h. Wells containing untreated cells (without addition of any test agents) were regarded as a negative control.

At the end of the incubation period, the Dulbecco's Modified Eagle's Medium (DMEM) of each well was replaced with DMEM medium containing 50 µg/mL Neutral Red (NR) solution and incubated for further 3 h to allow for uptake of the vital dye into the lysosomes of viable and uninjured cells. The medium was then removed and cells were rapidly washed with calcium chloride-formaldehyde mixture. The dye within viable cells was eluted from the cells with a mixture of acetic acid, ethanol and water (1:50:49) (0.2 mL). The plates were agitated on a micro titer plate shaker for 30 min and then absorbance ( $A$ ) was

measured at 540 nm using a micro plate reader. NR uptake, proportional to the number of viable cells within the well, was expressed as a percentage of uptake by control cells [ $(A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}}) \times 100 \%$ ].  $\text{IC}_{50}$  values (concentration required to reduce cells viability by 50 % as compared to the control cells) for each extract was extrapolated from the graphs plotted using the  $A$  values obtained.

**2,2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay** The electron donation abilities of the ACE of *G. mangostana* and *E. elatior* were measured according to the method described by Ge and Ma (2013) with a slight modification. 30 µL of ACE was mixed with 2 mL of 0.3 mM DPPH solution. After incubation for 30 min at room temperature (27°C) in darkness, the absorbance value of each mixture was measured at 517 nm by using cuvette and Thermo Scientific spectrophotometer (Fisher Scientific, Malaysia). The absorbance values recorded was used to calculate the scavenging rate for each concentrations of the sample. All the steps in this assay were run in triplicate. The scavenging rate of DPPH was calculated using the following formula:

$$\text{DPPH scavenging rate (\%)} = \frac{A_c - (A_i - A_j)}{A_c} \times 100$$

where  $A_c$  was the absorbance of DPPH methanolic alone;  $A_i$  was the absorbance of anthocyanin extract with DPPH methanolic; and  $A_j$  was the absorbance of anthocyanin extract without DPPH methanolic. The  $\text{IC}_{50}$  value (inhibition concentration that scavenges 50% of free radicals) for anthocyanin extracts of *E. elatior* inflorescence and ascorbic acid were determined by using the equation from their calibration curves.

**Ferric reducing antioxidant power (FRAP) assay** Briefly, the FRAP reagent was freshly prepared by using standard method as described by Peng and Zhao (2009). 50 µL of ACE of *E. elatior* were mixed with 1500 µL of FRAP reagent and further incubated for 30 min in water bath (37°C). The absorbance reading for each mixture then was measured at 593 nm. All the steps in this assay were run in triplicate.

#### DATA ANALYSIS

Data were expressed as mean  $\pm$  SD from three separate observations. For the antioxidant assay, the significance of difference was calculated by Student's  $t$  test and values  $<0.05$  were considered to be significance.

#### RESULTS AND DISCUSSION

##### TOTAL MONOMERIC ANTHOCYANIN CONTENT AND TOTAL PHENOLIC CONTENT (TPC)

The total monomeric anthocyanin content of ACE of *G. mangostana* and *E. elatior* were  $15.03 \pm 0.01$  and  $43.42 \pm 0.01$  mg/L ( $p < 0.05$ ), respectively (Table 1). The principle in pH-differential method is that the monomeric anthocyanin pigments undergo reversible structural

transformations with a change in a pH manifested by strikingly different absorbance spectra. The red coloured oxonium form predominates at pH1.0 and the colourless hemiketal form at pH4.5. This reaction permits an accurate and rapid measurement of anthocyanin, even in the presence of polymerized degraded pigment and other interfering compounds (Farah et al. 2008).

Our study also showed that ACE of *E. elatior* contains highest amount of phenolic, which is  $10.07 \pm 0.01$  g GAE/100 g FW, compared ACE of *G. mangostana*, which only contains  $3.29 \pm 0.01$  g GAE/100 g FW ( $p < 0.05$ ). Chan et al. (2007) reported that the TPC of *E. elatior* was 3.55 g GAE per 100 g FW. This species show the highest value in TPC when compared to other *Etilingera* species studied including *E. rubrostriata*, *E. littoralis* and *E. fulgens*. The difference in phenolic content of both *E. elatior* might be due to differences in plant parts used, source of plant and extraction methods.

#### EFFECT OF LIGHT, TEMPERATURE AND pH ON STABILITY OF ACE OF *G. MANGOSTANA* AND *E. ELATIOR*

**Temperature** Temperature is one of the most important factors that influence the stability of the anthocyanin during food processing and storage. The thermal stability study is essential to determine how long the anthocyanin can be kept in a particular temperature. The temperature chosen for this study was at  $99^\circ\text{C}$  to reflect a severe heat treatment during thermal food processing operations such as cooking

or pasteurization. Our study shows that the survival rate of ACE of *G. mangostana* drop faster compared to the survival rate of ACE of *E. elatior* after been exposed to high temperature in 120 min ( $p > 0.05$ ) (Figure 1). This data revealed that the anthocyanin of *E. elatior* could be more protective against thermal degradation compared with the anthocyanin in *G. mangostana*. The decrease in anthocyanin accumulation after being exposed to the higher temperature was resulted from anthocyanin degradation. The amount of anthocyanin found in plants decreases during processing and storage as temperature rises (Boranbayeva et al. 2014). Several studies also reported that the degradation rate of anthocyanin increased with increasing heating temperature (Hou et al. 2013; Solyom et al. 2014).

**Light** The stability of ACE of *G. mangostana* and *E. elatior* towards light was measured by colorant survival rate in absence or presence of light and shows that the stability of the ACE of *G. mangostana* was slightly affected ( $p > 0.05$ ) in the presence of light when compared to the *E. elatior* ( $p > 0.05$ ) (Figure 2). However, under the dark, the survival rate between these ACEs was more stable. Light is essential for the biosynthesis of anthocyanin, but at the same time it also accelerates their degradation or pigment destruction (Jaakola 2013). The longer anthocyanin being exposed to the light, the lower the anthocyanin content will be as the survival rate will be

TABLE 1. Total monomeric anthocyanin and total phenolic content, antioxidant assays and cytotoxicity of ACE

| Samples              | Anthocyanin (mg/L) | Phenolic (gGAE/100 g FW) | DPPH ( $\text{IC}_{50}$ , mg/mL) | FRAP (Abs at conc. range from 1.25 – 20 mg/mL) | Cytotoxicity on SKOV-3 cell ( $\text{IC}_{50}$ , $\mu\text{g/mL}$ ) |
|----------------------|--------------------|--------------------------|----------------------------------|------------------------------------------------|---------------------------------------------------------------------|
| <i>E. elatior</i>    | $43.42 \pm 3.47^*$ | $10.07 \pm 0.31^*$       | $14.90 \pm 0.09$                 | $0.31 \pm 0.04^* - 0.87 \pm 0.10^*$            | $54.32 \pm 4.60^*$                                                  |
| <i>G. mangostana</i> | $15.03 \pm 2.14^*$ | $3.29 \pm 0.44^*$        | $15.50 \pm 0.30$                 | $0.11 \pm 0.01^* - 0.14 \pm 0.01^*$            | $116.31 \pm 16.49^*$                                                |

Each value is the mean  $\pm$  standard deviation (S.D) consisting of three replicates.

GAE: gallic acid equivalent; FW: fresh weight; SKOV-3: human ovarian cancer cell line \* $p < 0.05$  were considered to be significantly difference

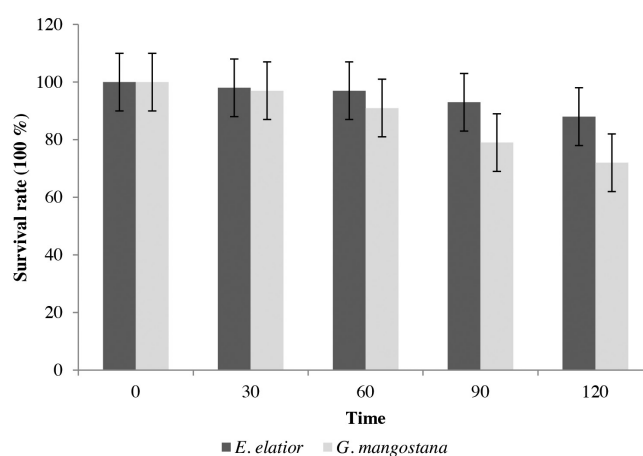


FIGURE 1. Effect of temperature on the stability of ACE of *G. mangostana* and *E. elatior*. ACEs were incubated in water bath at  $99^\circ\text{C}$  ( $\pm 1^\circ\text{C}$ ) for 0, 30, 60, 90 and 120 min. Each values represents mean  $\pm$  SD of three experiments

slowly decreased. Thus, the anthocyanin extracts should be kept away from the light during storage in order to maintain the colorant stability.

**pH** Effects of pH on maximum anthocyanin absorption at different period of time of the ACE of *G. mangostana* and *E. elatior* are shown in Figure 3. The different in pH highly influence the stability of the ACE of *G. mangostana* and *E. elatior* as the absorbance reading from the Day 1 to the Day 7 show a strong decrease ( $p > 0.05$ ). Anthocyanin is found to be more stable in acidic media at low pH values than in alkaline solutions with high pH values.

In general, below pH 2, anthocyanins were primarily in the form of the red flavylium cation. When pH increased more than 2, there will be a rapid proton loses favouring

red or blue quinonoidal forms. As the time pass by, the flavylium cation became hydrated to yield the colourless carbinol or pseudobase, which equilibrated to the open chalcone form and colourless (Marco et al. 2011).

#### BIOACTIVITY STUDIES

**Cytotoxicity studies** In the present study, ACE of *E. elatior* was found to have moderate cytotoxicity on human SKOV-3 ovarian cancer cell line with  $IC_{50}$  value of  $54.32 \pm 4.6 \mu\text{g/mL}$  ( $p < 0.05$ ) (Table 1). A literature survey revealed that there had been no detailed cytotoxic potential activity of ACE of *E. elatior* reported on human SKOV-3 ovarian cancer cell line. Aqueous extract of *E. elatior* had been reported to exert significant inhibition of cell proliferation on acute

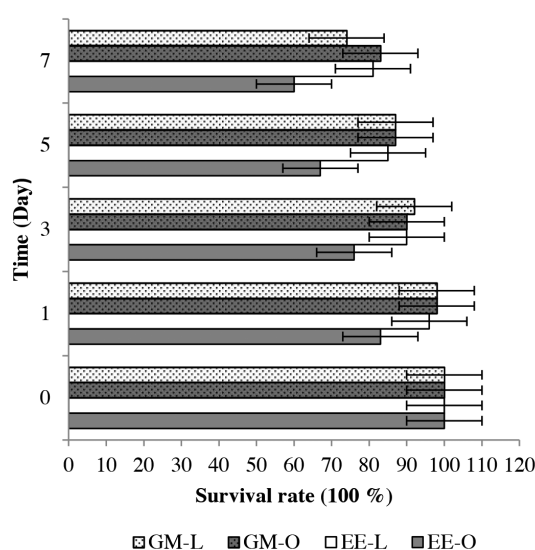


FIGURE 2. Effect of the absence (O) and presence (L) of light on the stability of the ACE of *G. mangostana* and *E. elatior*. GM: *G. mangostana*. EE: *E. elatior*. Each values represents mean  $\pm$  SD of three experiments

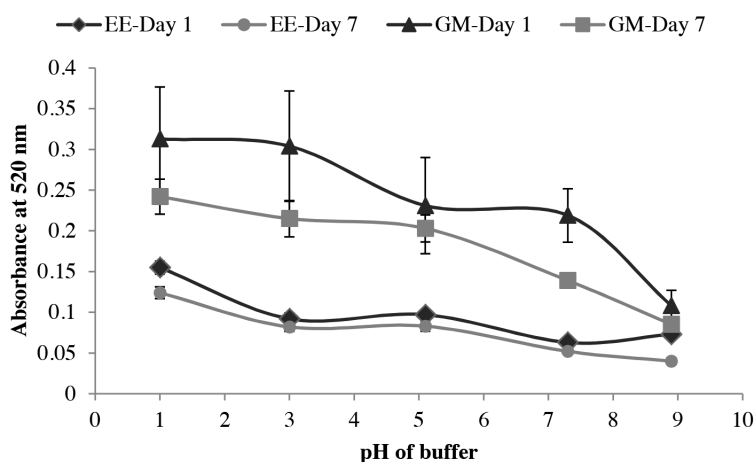


FIGURE 3. Stability of ACE of *G. mangostana* and *E. elatior* in different pH on Day 1 and Day 7. ACEs were incubated at 25°C, in different pH buffer and protected from light and oxygen. GM: *G. mangostana*. EE: *E. elatior*. Each values represents mean  $\pm$  SD of three experiments

TABLE 2. Pearson's correlation coefficients (r) between phytochemical content and bioactivity

| Pearson's correlation (r) |                      | DPPH   | FRAP at 20 mg/mL | Cytotoxicity |
|---------------------------|----------------------|--------|------------------|--------------|
| Anthocyanin               | <i>E. elatior</i>    | 0.473  | -0.537           | 0.326        |
|                           | <i>G. mangostana</i> | -0.674 | 0.739            | -0.994       |
| Phenolic                  | <i>E. elatior</i>    | 0.947  | 0.758            | 0.986        |
|                           | <i>G. mangostana</i> | -0.079 | -0.997           | 0.757        |

MV4-11 myeloid leukaemia and chronic K562 myeloid leukaemia cells (Jusoh et al. 2012).

**DPPH assay** In this study, DPPH assay was used to measure the potential free radical scavenger activities of ACE of *E. elatior* and *G. mangostana*. ACE of *E. elatior* demonstrated free radical scavenger effect with  $IC_{50}$  value of  $14.90 \pm 1.02$  mg/mL, meanwhile *G. mangostana* free radical scavenger effect was  $15.50 \pm 0.52$  mg/mL ( $p > 0.05$ ). The model of scavenging DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants. In the DPPH assay, the antioxidants are able to reduce the stable DPPH radical to the non-radical form DPPH-H. The DPPH scavenging activities of antioxidants attributed to their hydrogen donating abilities (Liu et al. 2013). It appears that both ACEs of *E. elatior* and *G. mangostana* possessed hydrogen donating abilities to act as antioxidant.

**FRAP assay** The reducing power of ACE of *E. elatior* and *G. mangostana* significantly increased with the increasing of their concentrations ( $p < 0.05$ ). ACE *E. elatior* shows a better increment in the reducing power activity compared to *G. mangostana*. Previous study by Chan et al. (2007) shows that among five of *Etilingera* species screened, *E. elatior* possessed the highest ferric reducing power. ACE of *E. elatior* exhibited higher antioxidant activities, compared to ACE of *G. mangostana*, in both antioxidant assays DPPH and FRAP assays which might be attributed with its high phenolic content. Flavonoids, terpenoids, saponins, tannins, carbohydrates, diarylheptanoids, labdane diterpenoids and steroids had been reported present in *E. elatior* (Lachumy et al. 2010).

**Correlation analysis** Correlation analysis was used to explore the relationships between the individual anthocyanin and phenolic compound and bioactivities studied for both ACE of *E. elatior* and *G. mangostana*. High positive correlation was observed between phenolic compound and all the biological activities in *E. elatior* (Table 2). Although positive correlations existed between antioxidant activity and phenolic compound in *E. elatior*, there was no correlation between antioxidant activities and phenolic compound of *G. mangostana*.

#### CONCLUSION

New sources of anthocyanin with high tinctorial power, stability and low cost are desired as natural food colorants.

Different parameters that can affect the stability of the anthocyanin such as the presence of oxygen, co-pigmentation, enzymes, concentration effects and others could be further study in order to establish the potential of anthocyanin from *E. elatior* and *G. mangostana* to be exploited as natural-based food colorant and as pharmaceuticals agent. The authors declare no conflict of interest.

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