International Food Research Journal 21(6): 2369-2375 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Anthocyanin content in relation to the antioxidant activity and colour properties of *Garcinia mangostana* peel, *Syzigium cumini* and *Clitoria ternatea* extracts

Siti Azima, A. M., *Noriham, A. and Manshoor, N.

Universiti Teknologi MARA, Department of Food Science and Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor

Article history

Received: 10 April 2013
Received in revised form: 6 May 2014
Accepted: 10 May 2014

Keywords

Anthocyanin
Antioxidant
Colour properties
Plants

Abstract

The plant extract serves not only as a good source of bioactive compounds but also as natural pigment that can be applied as colourants in food and pharmaceutical products. The aim of this study were to determine the anthocyanin content of *Garcinia mangostana* peel extract (GMPE), *Clitoria ternatea* extract (CTE) and *Syzigium cumini* extract (SCE) in relation to their antioxidant activity and their colour properties. The antioxidant activities related to the phenolic constituents including anthocyanin content were determined based on the EC_{50} of DPPH radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assay. The colour properties of the plant extracts were measured based on their degradation index (DI), indices of polymeric colour (PC) and colour density (CD). GMPE showed higher FRAP value and lower EC_{50} value which were 79.37 mmoles/g and 0.11 mg/ml, respectively, as compared to SCE extract with FRAP value, 25.66 mmoles/g and EC_{50} value, 0.22 mg/ml. Total monomeric anthocyanin (tmAcy) exhibited a strong correlation between FRAP assay (r^2 = 0.998) and DPPH assay (r^2 = 0.859). GMPE showed high CD (1.63 AU), moderate PC (0.18 AU) but low in DI (1.19 AU) while SCE exhibited low in CD (0.55 AU) and PC (0.07 AU) but moderate DI (1.26 AU). CTE exhibited high in DI (5.39 AU) and PC (0.19 AU) but moderate in CD (0.55). Hence, it can be concluded that colour pigment obtained from GMPE exhibited high antioxidant activity and better colour properties as compared to SCE and the strong correlation between tmAcy and two antioxidant activity assays which are FRAP and DPPH indicated that monomeric anthocyanin plays a major role in antioxidant activity of these plant extracts.

Introduction

The world is naturally decorated by the existence of colourful plants. The pigments that are responsible for the colour exist in the wide range of spectrum with different chromacity and hueness. Instead of being an ornamental to the nature their vibrant colours reward us with pharmacological and therapeutic properties. Plant pigments have gained a wide attention as good source of natural antioxidant which is associated with human well-being and health benefits. Owing to its phenolic structure, plant pigments are capable to overcome the oxidative stress in the human cell which finally led to the development of chronic disease such as tumour, cancer, neurodegenerative, rheumatic arthritis and autoimmune disorders (Velioglu *et al*., 1998; Goyal *et al.*, 2010). Recently, natural colourant from plant pigment are gaining marketplace in food colourants industry due to the banning of several synthetic food colourants including orange II, fast red, amaranth, auramine and rhodamine (Rao and Sudershan, 2005). Anthocyanin is a water soluble pigment ranging from orange, red, blue to violet chroma and it was scientifically claimed to possess antioxidant properties due to their chromophores structure of phenolic compounds (Delgado-Vargas *et al*., 2000; Hurtado *et al*., 2009). It has been classified under the phenolic pigment due to its structure of 2-phenylbenzopyrillium (Mercadante and Bobbio, 2008).

In food industry antioxidant was used to preserve the quality of a fatty food in relation to rancidity and off-odour. The unsaturated bonds present in fat represent an active site which may react with the oxygen and consequently lead to the formation of secondary and tertiary oxidation products that caused rancidity and loss of sensorial quality of food containing fat (Javanmardi *et al*., 2003; Huda-Faujan *et al*., 2007; Liu, 2010). The most commonly used antioxidants are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and ascorbic acid. Recently, the demand for natural phenolic antioxidant extracted from plants sources has gained a mountain interest to replace the synthetic

*Corresponding author.
Email: *noriham985@salam.uitm.edu.my*

antioxidant due to the toxicological issues caused by the synthetic ones. Phenolic compounds were able to counteract with the reactive oxygen species (ROS) due to their redox properties which allow them to act as reducing agent, hydrogen donators, singlet oxygen quencher and metal chelation potential (Huda-Faujan *et al*., 2009).

Phenolic compounds which generically denominated as ArOH contains at least one hydroxyl group (OH) attached to the benzene ring and plays as chain breaking antioxidant (Rojano *et al.*, 2008). Generally, there are two suggested mechanisms by which antioxidants can play their defensive role (Tachakittirungrod *et al.*, 2007; Wootton-Beard *et al.*, 2010). In the first mechanism known as hydrogen atom donator (HAT), the free radical removes a hydrogen atom from the antioxidant (ArOH) then becoming itself a radical and terminated the oxidation process by converting free radicals to more stable products (1). In the second mechanism SET (single-electron transfer), the antioxidant (ArOH) donates an electron to the free radical becoming itself a radical cation and terminate the oxidation chain reaction by reducing the oxidised intermediates into the stable form (2). Several methods have been established based on these two mechanisms. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, oxygen radical antioxidant capacity (ORAC) assay and total reactive antioxidant potential (TRAP) assay are categorised under the HAT while ferric reducing antioxidant power (FRAP), ferric thiocyanate (FTC) assay and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) scavenging assay are categorised under SET (Brand-Williams *et al*., 1995; Benzie and Strain, 1996; Ou *et al*., 2002).

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet} \quad (1)$$

$$R^{\bullet} + ArOH \rightarrow R^{-} + ArO^{\bullet+} \quad (2)$$

Mangosteen scientifically known as *Garcinia mangostana* was reported to contain numerous phenolic compositions including xanthone, phenolic acid and anthocyanin. Ten phenolics acid has been reported to be found in mangosteen fruits and protocatechuic acid was reported as the major phenolic acid being found in mangosteen peel and rind (Zadernowski *et al.*, 2009). The peel of the mangosteen was claimed to possess an antioxidant activity by scientific research and it has been traditionally used to treat several diseases. It has been reported to possess an antioxidant activity based on the DPPH radical scavenging assay, FRAP assay and ABTS assay (Okonogi *et al*., 2007; Pothitirat *et al.*, 2009; Khonkarn *et al.*, 2010). Mangosteen peel has been used by Asian people for treatment against diarrhoea, dysentery and cholera, to cure chronic intestinal catarrh and to heal skin infection and respiratory disorder (Milan and Osman, 2006). Instead of medicinal properties, mangosteen peel serves as the source of natural pigments which is anthocyanin. *Syzigium cumini* is a dark purple wild-berry being found in the tropical equatorial Asia. The dark purple of *Syzigium cumini* is due to the presence of anthocyanin (Lago *et al.*, 2004). Faria *et al.* (2011) reported that myricetin derivatives and gallic acid was identified in *Syzigium cumini*. It has been extensively used as a stomachic, astringent, antiscorbutic, diuretic, antidiabetic, and in chronic diarrhea in Ayurvedic medicine (Veigas *et al*., 2007). *Clitoria ternatea* with Double blue lines are dark blue in colour and it was reported to contain polyacylated anthocyanin (Kazuma *et al*., 2003). It has been traditionally used as a remedy for various diseases like urinogenital disorder, bronchitis, purgative, diuretic, rheumatism, anticancer and antidote for animal stings (Patil and Patil, 2011). The purpose of this study was to determine the anthocyanin content of *G. mangostana* peel, *Syzigium cumini* and *Clitoria ternatea* extracts in relation to their antioxidant activity and colour properties.

Materials and Methods

Chemical

Citric acid ($C_6H_8O_7$), gallic acid, quercetin, butylated hydroxy anisole (BHA), butylated hyroxy toluene (BHT), sodium citrate ($C_6H_5Na_3O_7$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), Aluminium chloride hexahydarate ($AlCl_3 \bullet 6H_2O$) and the Folin–Ciocalteu (FC) reagent were purchased from Sigma Chemical Co. (St. Louis, USA). Iron chloride hexahydrate ($FeCl_3 \bullet 6H_20$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6- tripyridyl-s-triazine (TPTZ). Potassium chloride (KCl) and sodium hydrogen phosphate (Na_2HPO_4) were purchased from Merck (Darmstadt, Germany). Sodium metabisulphite ($Na_2S_2O_5$) was purchased from APS Finechem (Sevenhill, Australia).

Sample preparation

The mangosteen peel was obtained from Sg. Siput, Perak, Malaysia and selected at 6th maturity stage based on the reference by Palapol *et al.* (2009). *Syzigium cumini* fruit and *Clitoria ternatea* flower were obtained from Jalan Santan, Perlis, Malaysia. Sample preparation was conducted based on Yang and Gadi (2008) with slight modification. The raw

materials were stirred in 100 mM citrate buffer (pH 3.0) for 10 minutes at 100°C. The ratio of plant parts to the buffer is 1:4 (v/v). The extracts were filtered by using Buchner funnel. The filtrate were collected and evaporated by using rotary evaporator at 60°C and 114 mbar. The concentrated filtrate were lyophilised and kept in amber bottle at 20°C prior to analysis.

Total monomeric anthocyanin

Total anthocyanin and monomeric anthocyanin in the extracted sample were measured spectrophotometrically according to the method described by Ciquanta *et al.* (2002) and Lee *et al.* (2005) with several modifications. Total anthocyanin content (tmAcy) was determined by single pH method at pH 1.0 while total monomeric content (tAcy) was determined by using pH differential method at pH 4.5 and pH 1.0. Approximately 10 mg of lyophilised plant extracts were extracted with 10 ml of HCl/water/ethanol solution (1/29/70, v/v/v). The extracts were centrifuged for 10 min at 10,000 g prior to the analysis. The degradation index (DI), indices of polymeric colour (PC), percent polymeric colour (%PC), colour density (CD), and colour tonality (CT) were conducted based on the method described by Giusti and Wrolstad (2005).

DPPH radical scavenging assay

Radical scavenging activity of the plant extracts were conducted based on the method developed by Brand-William *et al.* (1995) with slight modifications. Briefly, 600 µL of a various concentration of standard and tested sample ranging from 0-1 mg/ml were prepared depending on the optimum concentration of the sample to react with the DPPH molecules. Accurately, 4.5 mL of 50 µM DPPH solution in 95% ethanol was added to the sample solutions. The reaction was allowed to take place in the dark for 30 min and the absorbance reading at λ_{max} = 517 nm was recorded to determine the concentration of remaining DPPH. A blank corrected containing samples and ethanol without DPPH were also measured. The blank corrected is very important to avoid underestimation of the results obtained since anthocyanin absorbed the same wavelength with DPPH solution. The results were expressed as EC_{50} (efficient concentration at 50% scavenging activity). Ascorbic acid and a mixture of synthetic antioxidant BHA/BHT combination were used as standards in comparison with the extracts.

$$\% \text{ DPPH Radical scavenging activity} = \frac{\text{Absorbance blank control - (Absorbance sample - Absorbance blank corrected)}}{\text{Absorbance blank control}}$$

$$EC_{50} = \frac{(50 \quad c)}{m}$$

c = is the intercept of the graph
m = is the slope of the graph

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capability of the extract based on ferric reducing power was determined according to the method described by Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $Fe_2Cl_3 \cdot 6H_2O$ solution. The working solution was freshly prepared by mixing the acetate buffer, TPTZ solution and $Fe_2Cl_3 \cdot 6H_2O$ solution in 10:1:1 ratio and then incubated at 37°C for 10 minutes prior to analysis. 0.1 ml plant extracts were allowed to react with 2.9 mL of the FRAP solution for 30 minutes in the dark condition. The readings of the blue (ferrous tripyridyl-s-triazine) complex were measured spectrophometrically (Perkin Elmer UV-Vis spectrophotometer) at λ_{max} = 593 nm. The linear standard calibration curve ranging from 0-100 mM Trolox was established. The final results were expressed in mM TE/g of fresh extract weight.

Statistical analysis

Analyses of data were obtained from three triplicate samples. Statistical analyses were conducted using Statistical Analysis System 9.1.3 software package (SAS, 2002). Analysis of variance (ANOVA) in a completely randomised design and Duncan's multiple range tests were used to compare any significant differences between samples. Least significant difference (LSD) at 5% level was calculated to compare differences between means following a significant ANOVA effect. Values were expressed as means ± standard deviations.

Results and Discussion

Total anthocyanin and colour properties

Total anthocyanin indicates the total amount of anthocyanin including polymeric anthocyanin and anthocyanin degradation products while total monomeric anthocyanin indicates the amount of anthocyanin excluding polymeric anthocyanin and anthocyanin degradation product (Guisti and Jing, 2008). The basic concept of quantifying the anthocyanin content is the structural transformations of the anthocyanin based on the pH changes, in which the monomeric anthocyanin will exhibit a saturated red-bluish colour at pH 1.0 and colourless at pH 4.5 (Wrolstad *et al*., 2005). In contrast, the polymerised anthocyanin and other interfering compounds will retained their colour at pH 4.5. The changes of colour in respect of the structural transformation can be

Table 1. Total anthocyanin and colour properties of the pigmented plant extracts

Sample	tAcy (mg/g)	tmAcy (mg/g)	DI	CD	PC	%PC
GMPE	23.52 ± 0.32^{A}	19.82 ± 0.19^{A}	1.19 ± 0.02^{B}	1.63 ± 0.00^{A}	0.18 ± 0.02^{A}	11.45 ± 0.94^{C}
CTE	16.07 ± 0.02^{B}	2.98 ± 0.05^{C}	5.39 ± 0.08^{A}	1.01 ± 0.00^{B}	0.19 ± 0.02^{A}	18.35 ± 0.40^{A}
SCE	8.71 ± 0.14^{C}	6.92 ± 0.44^{B}	1.26 ± 0.07^{B}	0.55 ± 0.04^{C}	0.07 ± 0.02^{B}	13.23 ± 0.32^{B}

Note: Analysis of data was obtained from three triplicate samples.
^{ABC}Means with the different capital letter within the column were significant different at $p < 0.05$

Table 2. Antioxidant activities of the pigmented plants

Sample	DPPH radical scavenging assay, EC_{50} (mg/ml) [a]	FRAP assay (mM/g) TEAC
GMPE	0.11 ± 0.00^{C}	79.37 ± 0.77^{A}
CTE	0.49 ± 0.01^{A}	13.32 ± 0.28^{C}
SCE	0.22 ± 0.00^{B}	25.66 ± 1.40^{B}

[a] EC_{50} for Ascorbic Acid and BHA/BHT were 0.12 ± 0.00 and 0.10 ± 0.00 respectively
Note: Analysis of data was obtained from three triplicate samples.
^{ABC}Means with different capital letter within the column were significantly different at $p < 0.05$

Table 3. Correlations between anthocyanin content with the antioxidant activity and colour properties

Anthocyanin content	tAcy	tmAcy
DPPH	0.290	0.859
FRAP	0.775	0.998
DI	0.035	0.698
CD	0.996	0.790
%PC	0.761	0.205

quantified with the absorbance reading at the visible range of spectrophotometer with the correction of haze. The tAcy content of GMPE was 23.52 mg/g while SCE exhibited lower anthocyanin content which is 8.75 mg/g based on the cyanidin equivalent (Table 1). The amount of tAcy obtained for SCE was greater than the amount of tAcy (2.16 mg/g) in the *Syzygium cumini* peel extract reported by Veigas *et al.* (2007) and this could be due to different sample preparation employed and different part of sample which has been used. In the previous study mentioned, only peel has been used while for this study the whole fruit was used for the analysis. Other phenolic component from the flesh of *Syzygium cumini* might act as the copigment to preserve and stabilise the anthocyanin chromophores during the extraction and sample preparation.

Degradation index is defined as the ratio of the total anthocyanin to the total monomeric anthocyanin and the results were expressed in absorbance unit (AU). It has been extensively used to denote the amount of browning products in the anthocyanin containing product. CTE showed highest in DI (5.39) followed by SCE (1.26 AU) and GMPE (1.19 AU) exhibit lower DI. Anthocyanin naturally will condense with other phenolic compound to form a polymeric compound and this reaction is accelerated with the presence of acetaldehyde (Wrolstad *et al.*, 2005). Since the polymeric anthocyanin also absorbs at the maximum visible wavelength of the anthocyanin and it is pH insensitive so the measurement of browning by DI alone is not much applicable for the product containing polymeric anthocyanin (Giusti and Jing, 2008). In the case where the polymeric anthocyanins are presence the other parameter such as colour density and indices of polymeric should be counted for the quality judgement. The other characteristic that distinguished the monomeric and polymeric pigment is the reaction with bisulphite solution whereas the monomeric anthocyanin will react with bisulphite to form a colourless sulfonic compound. The justification to the stability of polymeric anthocyanins towards bisulphite reaction is due to the unavailability of the 4-position in the anthocyanin structure where it was covalently linked to another phenolic compound (Wrolstad *et al*., 2005). The indices of PC in the samples ranged from 0.07 to 0.19 AU with the highest PC being found in CTE and GMPE then followed by SCE. This denotes that the pigment presence in both CTE and GMPE are more stable than SCE in terms of insensitivity towards pH and bisulphite reaction. However, there was no significant different in indices of polymeric colour of CTE and GMPE at 5% confidence level. Colour density for all the samples ranged from 0.55 to 1.63 AU. The trend in CD followed the same trend as in tAcy in which the highest colour density showed by GMPE (1.63 AU) followed by CTE (1.01 AU) and the least CD was SCE (0.55 AU). This indicates that GMPE showed the most intense colour as compared to other samples and the intensity between all the samples were significantly differ at 5% confident level. This intensity might be due to the presence of anthocyanin and melanoidin since the trends were in agreement with the trend exhibited in tAcy. Percent polymeric colour of the samples ranged from 11.45% to 18.95% where the highest %PC found in CTE, followed by SCE and the least is GMPE. The highest indices of PC in CTE is in agreement with the previous study where *Clitoria ternatea* was reported to contain polyacylated delphinidin as the major pigment that was responsible for the deep blue colour of the flower (Kazuma *et al*., 2003; Kogawa *et al*., 2007). Thus, the acylation and glycosylation of anthocyanin may enhance the stability of anthocyanin (Cavalcanti *et al.*, 2011) and maybe to the extent of stability towards bisulphite reaction.

Antioxidant activity

DPPH scavenging activity is a kinetic antioxidant method which based on the reduction of DPPH$^{\bullet}$ free radical into $DPPH_2$ by the action of antioxidant (Miladorvic *et al.*, 2006). The $DPPH_2$ is a stable organic radical which exhibit violet colour in alcohol solution with an absorption peak in the visible range at λ_{max} = 518 nm and disappear in the presence of antioxidant. The capacity of the compounds to scavenge the DPPH radical has been used as the magnitude of the antioxidant capacity (Deng *et al*., 2011). This assay has been extensively used to assess antioxidant

activity in natural products, foods and beverages (Bortolomeazzi *et al*., 2007; Reddy *et al*., 2010). The EC_{50} for the sample ranged from 0.11 mg/ml to 0.49 mg/ml with the highest EC_{50} exhibited by CTE, followed by SCE and GMPE (Table 2). This indicates that GMPE is capable to scavenge 50% of the DPPH radical at the concentration of 0.11 mg/ml and exhibit the most efficient extract in DPPH radical scavenging activities as compared to the other samples. GMPE exhibited higher free radical scavenging activity as compared to the ascorbic acid. FRAP assay is based on the ability of the antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm (Yang and Zhai, 2010). FRAP values in the samples ranged from 13.32 mM/g to 79.37 mM/g based on trolox equivalent antioxidant capacity (TEAC). The trend was not similar as shown in TPC and TFC whereas the highest FRAP value showed in GMPE (79.37 mM/g) followed followed by SCE (25.66 mM/g) and the least FRAP value was showed in CTE (13.32 mM/g).

Correlations

The correlation analyses were conducted to determine the relationship between anthocyanin content and antioxidant activity as well as colour properties of the selected plant extract. From the results obtained in this work (Table 3), FRAP assay was found to be strongly correlated with tmAcy (r^2 = 0.998) and tAcy (r^2 = 0.775). The positive strong correlations between FRAP assay and tmAcy and between FRAP assay and tAcy complies with the previous work where positive strong relationship of anthocyanin extract with FRAP assay were reported (Sun *et al.*, 2009; Yang & Zhai, 2010).

The DPPH assay was found to be strongly correlated with tmAcy (r^2 = 0.859) and very weak correlation was observed with tAcy (r^2 = 0.290). The strong correlation between DPPH assay and tmAcy was in agreement with previous work by Yang and Zhai (2010). Based on chemistry structural point of view, monomeric anthocyanin possesses loose structures which are easier to undergo oxidation and thus will exhibit better antioxidant activity compared to the non-monomeric anthocyanin (Tsai & Huang, 2004; Wrolstad *et al*., 2005). This is also in agreement with Castaneda-Ovando *et al.* (2009) who claimed that the molecule which is easier to donate a free electron (Ionisation Potential (IP)) or hydrogen atoms (Bond Dissociation Energy (BDE)) to the reactive free radicals is often the best antioxidant, and increasing the stability of the anthocyanin will reduced its antioxidant stability. In details, they are easily counteracted with the lipid peroxidation by donating the H-atom to the peroxyl radical in the autoxidation thus act as the chain breaking antioxidant (Roginsky & Lissi, 2005). Otherwise, they are easily donating their electron and act as metal chelator or sequestrants by chelating the metallic ion such as copper (II) ion (Cu^{2+}) and iron (III) ion (Fe^{3+}) that promote the lipid peroxidation through catalytic action (Liu, 2010). This suggested that phenolic antioxidants are also able to hinder the propagation step by donating the electron to the unpaired valence shell electron of peroxyl radical ($ROO^{\bullet}$) and finally prevent the propagation of the autooxidation.

tAcy showed a very strong correlation (r^2 = 0.996) with CD while tmAcy exhibit strong correlation with CD which indicated that both parameter play a major role in colour properties of the plant extract. A strong correlation between tAcy and %PC and a negligible correlation between tAcy and DI can be observed which specified that tAcy is due to the polymerised anthocyanin but not the anthocyanin's degradation by-products (melainoidin and browning product). The unreliability of the DI in this study was confirmed by negligible correlation (r^2 = 0.035) between tAcy and DI while the reliability of the %PC was confirmed by strong correlation (r^2 = 0.761) between tAcy and %PC.

Conclusion

In both antioxidant activity assays studied, GMPE showed the highest antioxidant activity, followed by SCE and CTE. Monomeric anthocyanin plays a major role in their antioxidant activity based on the linear correlation coefficient. GMPE showed the highest colour density and the medium degradation index which indicates that GMPE would be the most potent extract that can be applied as natural colourant in foods, pharmaceutical and textile industry.

Acknowledgement

Special thanks to Universiti Teknologi MARA for funding this project under University Research Grant Scheme [600-RMI/DANA 5/3 PSI (323/2013)].

References

Benzie, I. F. F. and Strain, J. J. 1996.The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay. Analytical Biochemistry 239: 70–76.

Bortolomeazzi, R., Sebastianutto, N., Toniolo, R. and Pizzariello, A. 2007. Comparative evaluation of the

antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential. Food Chemistry 100: 1481–1489.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. LWT Food Science and Technology 28: 25-30.

Castaneda-Ovando, A., Pacheco-Hernandez, M. L., Paez-Hernandez, M. E., Rodriguez, J. A. and Galan-Vidal, C. A. 2009. Chemical studies of anthocyanins: A review. Food Chemistry 113: 859–871.

Cavalcanti, R. N., Santos, D. T. and Meireles. M. A. A. 2011. Non-thermal stabilization mechanisms of anthocyanins in model and food systems-An overview. Food Research International 44: 499–509.

Cinquanta, L., Matteo, M. D. and Estia, M. 2002. Physical pre-treatment of plums (*Prunus domestica*). Part 2. Effect on the quality characteristics of different prune cultivars. Food Chemistry 79: 233–238.

Delgado-Vargas, F., Jiménez, A. R. and Paredes-López, O. 2000. Natural Pigments: Carotenoids, Anthocyanins, and Betalains - Characteristics, Biosynthesis, Processing, and Stability. Critical Reviews in Food Science and Nutrition 40(3): 173-289.

Deng, J., Cheng, W. and Yang, G. 2011. A novel antioxidant activity index (AAU) for natural products using the DPPH assay. Food Chemistry 125: 1430–1435.

Giusti, M.M. and Jing, P. 2008. Analysis of Anthocyanin. In: Socaciu, C. editor. Food colorants chemical and functional properties, p. 480-497. Florida: Taylor and Francis Group.

Goyal, P. K., Verma, P., Sharma, P. and Parmar, J. and Agarwal, A. 2010. Evaluation of anti-cancer and anti-oxidative potential of *Syzygium cumini* against benzo[a]pyrene (BaP) induced gastric carcinogenesis in mice. Asian Pacific Journal of Cancer Prevention 11: 753-758.

Huda-Faujan, N., Noriham, A., Norrakiah, A.S. and Babji, A.S. 2007. Antioxidative activities of water extracts of some Malaysian herbs. ASEAN Food Journal 14 (1): 61-68.

Huda-Faujan, N., Noriham, A., Norrakiah, A.S. and Babji, A.S. 2009. Antioxidant activity of plants methanolic extracts containing phenolic compounds. African Journal of Biotechnology 8(3): 484-489.

Hurtado, N. H., Morales, A. L., González-Miret, M. L., Escudero-Gilete, M. L. and Heredia, F. J. 2009. Colour, pH stability and antioxidant activity of anthocyanin rutinosides isolated from tamarillo fruit (*Solanum betaceum* Cav.). Food Chemistry 117(1): 88-93.

Javanmardi, J., Stushnoff, C., Locke, E. and Vivanco, J. M. 2003. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chemistry 83(4): 547-550.

Kazuma, K., Noda, N. and Suzuki, M. 2003. Flavonoid composition related to petal colour in different lines of *Clitoria ternatea*. Phytochemistry 64: 1133-1139.

Khonkarn, R., Okonogi, S., Ampasavate, C. and Anuchapreeda, S. 2010. Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. Food and Chemical Toxicology 48(8–9): 2122-2129.

Kogawa, K., Kazuma, K., Kato, N., Noda, N. and Suzuki, M. 2007. Biosynthesis of malonylated flavonoid glycosidase on the basis of malonyl transferase activity in the petals of *Clitoria ternatea.* Journal of Plant Physiology 164: 886-894.

Lee, J., Durst, W. R. and Wrolstad, R.E. 2005. Determination of total monomeric anthocyanin pigment Content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative Study. Journal of AOAC 88 (5): 1269-1278.

Liu, Z. Q. 2010. Chemical methods to evaluate antioxidant ability. Chemical Review 110: 5675-5691.

Milan, A. R. and Osman, M. 2006. Fruits for the future 9: Mangosteen *Garcinia mangostana*: Crops for the Future, p. 1-13, 55-59. England : Southampton Centre for Underutilised Crops.

Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S. and Chowwanapoonpohn, S. 2007. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chemistry 103(3): 839-846.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. and Deemer, E. K. 2002. Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays: A comparative study. Journal of Agricultural and Food Chemistry 50: 3122-3128.

Palapol, Y., Ketsa, S., Stevenson, D., Cooney, J. M., Allan, A. C. and Ferguson, I.B. 2009. Colour development and quality of mangosteen (*Garcinia mangostana* L.) fruit during ripening and after harvest. Postharvest Biology and Technology 51: 349–353.

Patil, A. P. and Patil, V. R. 2011. *Clitoria ternatea* Linn.: An Overview. International Journal of Pharmaceutical Research 3(1): 20-23.

Pothitirat, W., Chomnawang , M. T., Supabphol, R. and Gritsanapan, W. 2009. Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the mangosteen fruit rind at two stages of maturity. Fitoterapia 80: 442–447.

Rao, P., and Sudershan, R. V. 2008. Risk assessment of synthetic food colours: a case study in Hyderabad, India. International Journal of Food Safety, Nutrition and Public Health 1(1): 68-87.

Reddy, C. V. K., Sreeramulu, D. and Raghunath, M. 2010. Antioxidant activity of fresh and dry fruits commonly consumed in India. Food Research International 43 (1): 285–288.

Roginsky, V. and Lissi, E. A. 2005. Review of methods to determine chain-breaking antioxidant activity in food. Food Chemistry 92(2): 235-254.

Rojano, B., Saez, J., Schinella, G., Quijano, J., Ve´lez, E., Gil, A. and Notario, R. 2008. Experimental and theoretical determination of the antioxidant properties

of isoespintanol (2-isopropyl-3,6-dimethoxy-5-methylphenol). Journal of Molecular Structure 877: 1–6.

Sun, J., Yao, J., Huang, S., Long, X., Wang, J. and García-García, E. 2009. Antioxidant activity of polyphenol and anthocyanin extracts from fruits of *Kadsura coccinea* (Lem.) A.C. Smith. Food Chemistry 117: 276–281.

Tachakittirungrod, S., Okonogi, S. and Chowwanapoonpohn, S. 2007. Study on antioxidant activity of certain plants in Thailand: Mechanism of antioxidant action of guava leaf extract. Food Chemistry 103: 381–388.

Tsai, P. J. and Huang, H. P. 2004. Effect of polymerization on the antioxidant capacity of anthocyanins in Roselle. Food Research International 37: 313–318.

Veigas, J. M., Narayan, M. S., Laxman, P. M. and Neelwarne, B. 2007. Chemical nature, stability and bioefficacies of anthocyanins from fruit peel of *Syzygium cumini* Skeels. Food Chemistry 105: 619–627.

Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 46: 4113–4117.

Wootton-Beard, P. C., Moran, A. and Ryan, L. 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. Food Research International 44: 217- 224.

Wrolstad, R. E., Durst R. W. and Lee, J. 2005. Tracking color and pigment changes in anthocyanin products. Trends in Food Science and Technology 16: 423–428.

Yang, J. and Gadi, R. L. 2008. Effect of steaming and dehydration on Anthocyanin. Antioxidant Activity, Total Phenols and color characteristic of Purple-Fleshed Sweet Potatoes (*Ipomeoa batatas*). American Journals of Food Technology 4: 224-238.

Yang, Z. and Zhai, W. 2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innovative Food Science and Emerging Technologies 11: 169–176.

Zadernowski, R., Czaplicki, S. and Naczk, M. 2009. Phenolic acid profiles of mangosteen fruits (*Garcinia mangostana*). Food Chemistry 112(3): 685-689.