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PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF SELECTED PIGMENTED PLANTS: *Garcinia mangostana*, *Clitoria ternatea*, *Ardisia colorata* var *elliptica* and *Syzygium cumini*

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Abstract

In recent years, the application of plant extract as natural antimicrobial and colourants in food are gaining interest, concurrently with the increasing reports and consumer awareness on the adverse effect of the synthetic ones. Hence, the objectives of this study were to evaluate the phytochemicals and antimicrobial activity of the selected pigmented plants namely, *Garcinia mangostana* peel, *Clitoria ternatea* flower, *Ardisia colorata* var *elliptica* fruit and *Syzygium cumini* fruit. The phytochemicals present in the selected pigmented plants were determined based on the phytochemical screening and liquid chromatography mass spectrometry quadrupole time-of-flight (LC-MS Q-TOF), while antimicrobial activity analysis was carried out using the disc diffusion technique. Phytochemical screening revealed that *Garcinia mangostana* exhibited the strongest indications for the presence of flavonoid, leucoanthocyanidin, quinone, tannin and anthocyanidin than other pigmented plants studied. Three anthocyanin namely, delphinidin-3-O-glucoside, delphinidin-3-O-galactoside and malvidin-3-O-galactoside was first time identified in *A. colorata* by LC-MS Q-TOF. The antimicrobial activity analysis showed that, *Garcinia mangostana* peel exhibited the strongest inhibition on fungi *Aspergillus niger* and gram-positive bacteria i.e. *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* with minimum inhibitory zone of 6.50, 8.50, 6.70, and 7.20 mm, respectively. Therefore, our results suggested that the antimicrobial activity of *Garcinia mangostana* peel was associated with their specific phenolic compounds. From a practical point of view, *Garcinia mangostana* peel may be a good candidate for functional foods and pharmaceutical applications.

Keywords: anthocyanin, antimicrobial activity, LC-MS Q-TOF, phytochemical, pigmented plant

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Introduction

Currently, there is an increased global interest on the phytochemicals due to the recognition of their nutraceutical and medicinal values to human. Phenolic compounds are plant secondary metabolites which play an important role in the physiological balance of plants, counteract with the biological stress and repel or attract other organism (Bernal *et al.*, 2011). These may include flavonol, flavanols, flavanones, anthocyanins, betalains, alkaloids, terpenoids, steroids, quinones and saponins. Numerous researches have been conducted on the antimicrobial activities of plant extracts related to their phenolic compounds. Previous studies reported on the antimicrobial activities of ethanolic and methanolic extracts of *Garcinia mangostana* peel (Geetha *et al.*, 2011), aqueous extract of *Clitoria ternatea* flower (Uma *et al.*, 2009), non-aqueous extract of *Syzygium cumini* fruit peel extract (Priya *et al.*, 2013), and leaves and bark of *Ardisia colorata* (Syed *et al.*, 2013). However, lack of study been conducted on the antimicrobial activitiy of the aqueous extract of *A. colorata* fruits, *S. cumini* fruits, and *G. mangostana*

peel.

In recent years natural colourants from plants are gaining interest due to the increasing reports of toxicity, hyperactivity in children and allergic reaction of synthetic additives and insect derived-colourants (Biswas *et al.*, 2020; Cox *et al.*, 2012; McCann *et al.*, 2007). In response to this issue, the United Kingdom Food Standard Agency (UK FSA) recommended the food manufacturers to find the alternatives for six artificial colourings named as '*The Southampton Six'* and these include Tartrazine, Ponceau 4R, Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red which are commonly found in sweets, biscuits, and soft drinks. Concurrent with the emerging of *'halal'* (Malay word) industry and increasing reports on the adverse effect of synthetic and animal-derived colourants on human, the applications of anthocyanin in food are gaining interest among the food researchers and manufactures in replace of synthetic and animal-derived colourants. Although the usage of synthetic colourants has gained critics globally, the awareness on the adverse effect of the synthetic colourants among Malaysian is lacking. Indeed, the emerging of "rainbow foods" such as '*laksa pelangi'*, *'roti canai pelangi'* indicates that Malaysian are getting more excited on colouring their (Husain, 2014; Hussin, 2015; Samichu, 2016).

In addition, anthocyanin has a promising potential as a substitute to several banned food colourant due to their compatibility in hue and chroma to Allura Red. In recent years, utilization of plant extract as colourants have gained a huge interest in food industry in replace of synthetic colourants which is proven toxic and detrimental to human (Arnold *et al.*, 2012; Biswas *et al.*, 2020). The identification and quantification of anthocyanin in *G. mangostana*, *C. ternatea* and *S. cumini* have been reported by several authors, however the report on anthocyanin in *A. colorata* var *elliptica* is scarce. Thus, the main objectives of this study were to determine the phytochemicals and antimicrobial activity of the selected pigmented plants. The identification of phenolic compounds and anthocyanin in the selected pigmented plants was determined by liquid chromatography mass spectrometry quadrupole time-of-flight (LC-MS Q-TOF).

Methods

Sample Preparation

Garcinia mangostana fruits were collected from two mangosteen trees at Sg. Siput, Perak, Malaysia and the fresh mangosteen fruits were selected for uniformity of colour at the maturity index 6. The *Clitoria ternatea* flower was obtained from four *C. ternatea's* trees at Santan, Perlis, Malaysia. The *C. ternatea* flower was selected for uniformity at the full bloom stage (stage 4). The *Syzygium cumini* fruit was collected from a tree from Dato' Kayaman, Perlis, Malaysia. The *S. cumini* fruits were selected for uniformity of colour at the maturity index 4. The *Ardisia colorata* var. *elliptica* fruit was collected from two *A. colorata*'s trees at Santan, Perlis, Malaysia. The *A. colorata* fruits were selected for uniformity of colour at the maturity index 4. The identification and voucher number for *Clitoria ternatea* (SK 2108/13), *Syzygium cumini* (SK 2110/13) and *Ardisia colorata* Roxb. var. *elliptica* King & Gamble (SK 2109/13) were obtained from Herbarium Institute Biosains, Universiti Putra Malaysia (UPM), Serdang, Malaysia.

The samples were packed in Low-Density Polyethylene (LDPE) zipper-lock plastic packaging and stored in the ice box with the controlled temperature (4°C) for approximately 7 hours during transportation to Universiti Teknologi MARA, Shah Alam Selangor, Malaysia. The samples were stored less than 2 weeks at -20°C in a freezer before the extraction process. The samples were thawed for 30 minutes at the room temperature (25°C) before the extraction. Prior to the extraction, the seed from *S. cumini* fruit was separated from the other part, where only the skin and flesh were used as the seed may contribute to the astringency of the extract.

Extraction procedure

The preparation of raw materials and the aqueous extraction procedure of *G. mangostana* peel, *C. ternatea* flower, *A. colorata* var. *elliptica* and *S. cumini* fruits was conducted according Siti Azima *et al.,* (2017). Briefly, the sample was stirred in distilled water at 100°C for 10 minutes. The ratio of water

to plant part is 1:4 (w/v). The residue was subsequently extracted twice with distilled water and the extracts were combined. The extracts were filtered using a Whatman No 1 filter paper and Buchner funnel connected with a vacuum pump (Gast DAA-V715A-EB, Vatech, Malaysia). The filtrate was collected and vapourised using a rotary evaporator (Buchi R-210, Switzerland) at 60°C and 114 mbar. The concentrated filtrate was lyophilised using a freeze dryer (Christ, Alpha 1-4 LD Plus, Germany) at -55°C and 0.056 mbar, equipped with RZ 2.5 vacuum pump.

Phytochemicals Screening

The phytochemical screening of the lyophilised extract were conducted according to the method as described in Ayoola *et al.,* (2008) and Guerrero *et al.,* (2012).

Flavonoid Test. About 0.5 g of the powdered samples was dissolved in 20 ml of water in a test tube. Approximately, 0.5 g magnesium filling and followed by 2 drops of 1.2 M HCl were added to the mixture. Colours ranging from orange to red indicated the presence of flavones, red to crimson indicated flavonols and crimson to magenta indicate flavonones. The estimated quantity was qualitative evaluated based on the intensity of colour formed by the reaction of samples and reagent.

Saponin Test. Approximately, 0.5 g of the powdered sample was dissolved in 20 ml of distilled water. Accurately, 10 ml of the sample's solution was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion. The stable persistent froth indicated the presence of saponins. The estimated quantity was qualitative evaluated based on the level of froth formed by the reaction of samples and reagent.

Tannin Test. About 0.5 g of the powdered samples was dissolved in 20 ml of water in a test tube. Two drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration. The estimated quantity was qualitative evaluated based on the intensity of brownish green or a blue-black colour formed by the reaction of samples and reagent.

Quinone Test. Approximately 0.5 g of the dried powdered samples was dissolved in 20 ml of water in a test tube. About 1 ml of 20% H_2O_2 and 1 ml of 50% H_2SO_4 were added to the filtrate. After heating in the water bath for 5 minutes, 5 ml of toluene was added to the filtrate and then shook vigorously. Then 5 ml of 5% NaOH was added to the toluene phase. The presence of red colour on the toluene phase was regarded as the presence of quinones. The estimated quantity was qualitative evaluated based on the intensity of red colour formed by the reaction of samples and reagent.

Detection of Anthocyanin. About 0.5 g of the dried powdered samples was dissolved in 20 ml of water in a test tube and then divided into two other test tubes where each test tube containing of 5 ml of the sample solution. Accurately, 5 ml of the 0.025 M potassium chloride (pH 1.0) were added to the first test tube and 5 ml of 0.4 M sodium acetate (pH 4.5) were added into the second test tube. The difference of colour in both reactions indicated the presence of anthocyanin while the unchanged in the colour tint for both reactions indicated the presence of betalain. The estimated quantity was qualitative evaluated based on the intensity of remaining colour after the reaction.

Detection of Leucoanthocyanidin. About 0.5 g of the dried powdered samples was dissolved in 20 ml of water in a test tube. About 5 ml of isoamyl alcohol was added to the 5 ml of the extract. Then the extract was treated with concentrated HCl and any colour change was observed. The solution was transferred in an encapped boiling tube and boiled for 15 min. The presence of red or pink tint in the upper layer of the solution indicated the presence of leucoanthocyanidin. The estimated quantity was qualitative evaluated based on the intensity of red or pink tint in the upper layer of the solution.

Alkaloid Test. About 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. Then, 2 ml of diluted ammonia was added to 5 ml of the filtrate. About 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic

acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish-brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids. The estimated quantity was qualitative evaluated based on the intensity of cream or reddish-brown colour formed by the reaction of samples and reagent.

Liquid Chromatography Analysis

The solid phase extraction (SPE) was conducted according to Siti Azima *et al.* (2017). Accurately, 0.5 g extract was dissolved in 20 ml of acidified deionised water (adjusted to pH 2.0 with HCl) and stirred using a magnetic stirrer for 15 min. Extraction of phenolic compounds was performed with Vacuum Manifold Processing Station (Agilent Technologies Inc, USA). A Sep-Pak C18 cartridge (Waters, Massachusetts, USA) was conditioned with 20 ml of methanol through sorbent bed. Approximately, 30 ml of acidified water (0.01% HCl) were passed through the cartridge to remove the remaining methanol. Then, the sample was passed through the cartridge and washed with 50 ml of acidified water to remove all the sugars. The adsorbed compound was eluted with ethyl acetate (50 ml) to obtain the non-anthocyanin phenolic compounds. The anthocyanin was eluted with acidified methanol (0.01% HCl). The extract was concentrated under reduced pressure at 40°C until constant weight was obtained. The phenolic extract was diluted to 20 ml with 50% methanol while the anthocyanin extract was diluted to 50 ml with 50% acidified methanol (0.1% HCl). Then the extract was filtered through a 0.45 µm membrane filter and injected into a LC-MS Q-TOF system.

Meanwhile, for the LC-MS Q-TOF, the chromatographic separation was performed by Liquid Chromatography of 1200 Rapid Resolution Series (Agilent Technologies, Santa Clara, CA, USA) consisting of a binary pump, degasser, 96-well plate autosampler with thermostat, thermostat column compartment, 6520 Q-TOF mass spectrometer equipped with a dual electrospray ionization (ESI) source and column used was Poroshell 120-Reverse Phase column (2.7 µm, 2.1 × 100 mm, Agilent Technologies Inc., USA). The solvent system used was as according to method as described in Siti Azima *et al.* (2017). The nebulizer was set at 45 psig and the drying gas nitrogen was set at a flow rate of 12 l/min. The drying gas temperature was maintained at 350°C. The samples were run in positive mode. Internal reference ions were used to correct mass accuracy. Autocalibration parameters were chosen to average five scans and reference mass correction was enabled throughout the run. The external standards cyanidin 3-sophoroside, cyanidin 3-glucoside, malvidin 3-glucoside, delphinidin and cyanidin at 0.5 mg/ml were used as reference standard. The peaks in the sample's chromatogram were compared with retention time and *m/z* from the external standard. While the *m/z* of unidentified peaks we compared with the available literatures.

Antimicrobial Analysis

Antimicrobial activities of the plant extracts were conducted based on the method described in Mahesh *et al.* (2008), and the test microorganisms were obtained from the Microbiology Culture Laboratory, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia. The test microorganisms consisted of Gram-positive *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*; Gram-negative *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. The fungi used were the yeast *Candida albicans* and the mold *Aspergillus niger*.

The bacteria were pre-cultured in NB for 24 hours at 37°C in the waterbath shaker and then adjusted to $O.D_{610nm}$ at 0.10 ± 0.02 AU using a spectrophotometer (Secomam, Prim Light 230 VAC, France). As for the *C. albicans* the yeast was cultured in PDB for 3 days at 37°C and the suspension was adjusted spectrophotometrically to $O.D_{530nm}$ to 0.10 ± 0.02 AU. While, fungal inoculum of *A. niger* was prepared from 5 days old culture grown in potato dextrose agar medium. The petri dish was flooded with 10 ml of distilled water and the conidia was scraped using sterile spatula. The spore density of each fungus was adjusted to $O.D_{595nm}$ 0.10 ± 0.02 AU to obtain a final concentration of approximately 1×10^5 spores/ml. Then the agars were inoculated simultaneously in three directions using a sterile swab dipped in the diluted suspension and allowed to dry for 15 to 30 min.

The discs which had been impregnated with 25 µL of a series concentration (12.5-100 mg/ml) of plant extracts were placed on the nutrient agar and potato dextrose agar surface. The plates were incubated at 37°C for 18–24 h for bacterial pathogens and 3 days for fungal pathogens in an incubator (Memmert, Germany). Chloramphenicol (0.03 mg/disc) and penicillin (0.06 mg/disc) were used as standards antibacterial while nystatin (0.1 mg/disc) was used as a standard antifungal. The commercial standard antimicrobial discs were purchased from Oxoid, UK.The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was carried out in triplicate and the mean of the diameter of the inhibition zones was calculated.

Statistical Analysis

The statistical analysis was conducted by using SAS (2002). The analyses of data were obtained from three triplicate samples (n=3). Values were expressed as means ± standard deviations (SD).

Result and Discussion

Phytochemical Screening

Phytochemical screening of the selected pigmented plants revealed that the entire sample contained flavonoids, anthocyanidin leucoanthocyanidin, and quinones as shown Table 1. All samples except *C. ternatea* flower indicated the presence of tannins. Though, the entire sample exhibited a negative indication for the presence of alkaloids and saponins. Previous literature also reported the presence of tannins in *G. mangostana* peel (Suttirak *et al.*, 2014) and *S. cumini* fruit (Zhang *et al.*, 2009). On contrary, Mathew *et al.,* (2009) reported the presence of tannins in *C. ternatea* flower extracts. The deviation between the results of this study and Mathew *et al.* (2009) could be due to the difference in the type of solvent used in the extraction procedure of *C. ternatea* flower. In Mathew *et al.,* (2009), methanol was used as the extractant while in this study water was used as the extractant. Type of solvent may affect the type of the extractable phenolic compounds and its bioactivity since each phenolic compound has a different affinity towards polarity of solvent (Aires, 2017; Iloki-Assanga *et al.*, 2015). Moreover, genetic and genomic variations may affect the diversification of phytochemical presence among the samples and within the similar sample of different study (Imeh *et al.*, 2002; Tavarini *et al.*, 2008).

Ferrara *et al.,* (1989) claimed that the toxicity of pomegranate fruit is due to the presence of alkaloids. Thus, the absence of alkaloids in the selected pigmented plant extract obtained by water extraction may hypothetically suggest the non-toxicity of these plants; however, a toxicological study should be conducted to confirm the toxicological status of these plants. The negative indication of alkaloids was also reported in methanolic extract of *C. ternatea* flower (Mathew *et al.*, 2009). Abuzaid *et al.,* (2018) also reported the negative indication of alkaloids in 50% aqueous ethanol of *G. mangostana* peel extract. However, a traces concentration of alkaloids was previously reported in ethanolic extract of *A. colorata* fruits, barks and leaves (Phatthalung *et al.*, 2012).

Flavonoids possess numerous medicinal properties including antioxidant, antiviral, antifungal, anticarcinogenic and anti-inflammatory activities (Okamura *et al.*, 1993). Flavonoids were found to be the major phytochemical constituent in *G. mangostana* peel, *C. ternatea* flower, *A. colorata*, *S. cumini* fruits. High concentration of flavonoid in *G. mangostana* peel may emphasise the medicinal uses of *G. mangostana* and its commercialisation as health drinks. Manimekalai *et al.,* (2016) also reported the presence of flavonoids and a negative indication of saponins in *G. mangostana* peel. Meanwhile, *S. cumini* fruit was reported to be rich in flavonoids (Ayyanar *et al.*, 2013). Flavonoids were also identified in the petal of *C. ternatea* (Kazuma *et al.*, 2003a; Kazuma *et al.*, 2003b). However, Phatthalung *et al.,* (2012) reported a negative indication of flavonoids in the ethanolic *A. colorata* fruits extract. Perhaps, the affinity of flavonoids presence in *A. colorata* towards more polar solvent (water) as compare to less polar solvent (ethanol) caused the deviation between the presence of flavonoids in this study and the absence of flavonoids in the previous study by Phatthalung *et al.*, (2012) (Aires, 2017; Iloki-Assanga *et al.*, 2015). The difference in agronomic, genomic, pre- and post-harvest factors might also contribute to the deviation between the extractable phytochemical compounds found in this study and the previous study (Imeh *et al.*, 2002). The highest concentration of flavonoids in *G. mangostana* and the presence of flavonoid in all the tested samples is in line with our previous study in Siti Azima *et al.,* (2017)

which conducted on the determination of flavonoids by RP-HPLC.

Table 1. Phytochemical screening of the selected pigmented plants

Plant sample	Secondary metabolites	Estimated Quantity
G. mangostana peel	Flavonoid	+++
	Leucoanthocyanidin	+++
	Quinone	+++
	Tannin	+++
	Anthocyanidin	+++
	Alkaloid	-
	Saponin	-
C. ternatea flower	Flavonoid	++
	Leucoanthocyanidin	+
	Quinone	++
	Tannin	-
	Anthocyanidin	+
	Alkaloid	-
	Saponin	-
S. cumini fruit	Flavonoid	+
	Leucoanthocyanidin	++
	Quinone	++
	Tannin	++
	Anthocyanidin	++
	Alkaloid	-
	Saponin	-
A. colorata fruit	Flavonoid	++
	Leucoanthocyanidin	++
	Quinone	++
	Tannin	+
	Anthocyanidin	++
	Alkaloid	-
	Saponin	-

Note: Symbols (+++) indicates the presence of phytochemical constituent in high concentration, (++) indicates the presence of phytochemical constituent in moderate concentration, (+) indicates the presence of phytochemical constituent in trace concentration and (-) indicates the absence of phytochemical constituent.

Anthocyanidins and leucoanthocyanidin were found in all tested sample with the highest indication in *G. mangostana* peel followed by *S. cumini* fruit, *A. colorata* fruit, and *C. ternatea* flower. Previous study also reported the presence of anthocyanin in *G. mangostana* peel (Palapol *et al.*, 2009), *C. ternatea* flower (Kazuma *et al.*, 2003b) and *S. cumini* fruit (Faria *et al.*, 2011; Jampani *et al.*, 2014; Veigas *et al.*, 2007). Up to date, there is no study reporting on the anthocyanin in *A. colorata*, however the presence of anthocyanin in *A. compressa* was reported by Joaquín-Cruz *et al.* (2015), which share the same genus and having closely physical resemblance to each other. Our previous study in Siti Azima *et al.*, (2017) conducted on the determination of anthocyanin by RP-HPLC confirmed the presence of anthocyanin in all the tested samples.

Leucoanthocyanidins (flavan-3,4-diols) are monomeric flavonoids that produce anthocyanidins by cleavage of a carbon-oxygen bond on heating with mineral acid. Leucoanthocyanidin is also known as a precursor in the biosynthesis of anthocyanin and proanthocyanidin (Reddy *et al.*, 2007). The reaction leading from colourless leucoanthocyanidin to anthocyanidin and its 3-glucoside is the critical step in the formation of coloured metabolites in the synthesis of anthocyanin (Nakajima *et al.*, 2001). The result from this study indicated that *G. mangostana* peel showed a high concentration of leucoanthocyanidins, *A. colorata* and *S. cumini* fruits showed a moderate concentration of leucoanthocyanidins while *C.*

ternatea flower showed traces concentration of leucoanthocyanidin. The presence of proanthocyanidins in *A. colorata* fruit (Phatthalung *et al.*, 2012), *G. mangostana* peel (Fu *et al.*, 2007; Zarena *et al.*, 2012) and *C. ternatea* flower (Nair *et al.*, 2015) as reported in the previous literatures could be related to the presence leucoanthocyanidin in the samples obtained in this study. Previous studies reported a strong correlation between leucoanthocyanidin content with FRAP and DPPH radical scavenging assays (Maksimović *et al.*, 2005; Malenčić *et al.*, 2008).

Based on the screening of quinones, *G. mangostana* peel was found to have a strong indication of quinones. *C. ternatea* flower, *S. cumuni* fruit, and *A. colorata* fruit exhibited moderate indication of quinones. Pradipta *et al.,* (2007) also reported the presence of quinones in *G. mangostana* peel. Quinones are the largest group of natural pigments which are widely distributed in higher plants, fungi, lichens and some vertebrates. It can be divided into three major groups which are benzoquinones, naphthoquinones and anthraquinones (Pintea, 2007). Benzoquinones showed a pink hue at high concentration while the predominant colour of naphthoquinone may vary from red-blue-green colour and anthraquinones is responsible for the red-purple hue of carminic (Delgado-Vargas, 2003). Pyrroloquinoline (PPQ) is a derivative of quinones and it is gaining interest due to its remarkable antioxidant properties as well as growth-promoting factor while carminic acid derived from the insect-based, is a type of quinone that is widely used as colouring in food (Magnusson *et al.*, 2004; Rucker *et al.*, 2000). Lack of study reporting the presence quinones in *A. colorata*; however, the presence of ardisiaquinones was reported in *A. seiboldii* (Ogawa *et al.*, 1968) and *A. teysmanniana* leaves (Yang *et al.*, 2001).

Phenolic Compounds

LC-MS Q-TOF was performed to identify the mass spectra and *m/z* ratio of the compound corresponding to the peak. Based on Electrospray Ionisation Total Ion Chromatogram (ESI TIC) of LC-MS Q-TOF as shown in Figure 1, there were some peaks that could not be identified by the standard used. Since pure standards are expensive and are about hundreds of anthocyanin derivatives make it almost impossible for the identification of the peak. Therefore, the unknown anthocyanins were identified by comparing the $[M+H]^+$ *m/z* ratio to the published value. Table 2 shows the retention time and *m/z* ratio of the identified compounds by LC-MS Q-TOF.

As shown in Figure 1 *(a)* and Table 2 peak [1] and [2] with $[M+H]^+$ *m/z* 611 and 449 could be identified as cyanidin-3-O-sophoroside and cyanidin-3-O-glucoside, respectively. Correspondingly, this finding supported our previous findings in Siti Azima *et al.* (2017), on the identification of cyanidin-3-O-sophoroside and cyanidin-3-O-glucoside in *G. mangostana* by RP-HPLC. The presence of cyanidin-3-O-sophoroside and cyanidin-3-O-glucoside as the major pigments in *G. mangostana* peel with molecular ion peak at *m/z* 611 and 449, respectively were also identified by Palapol *et al.* (2009) and Zarena and Udaya Sankar (2012).

Based on the $[M+H]^+$ *m/z* obtained from Q-TOF and anthcocyanin chromatogram of *Clitoria ternatea* shown in Figure 1 *(b)* and Table 2, peak [1] and [2] could be assigned as delphinidin derivatives. Nair *et al.*, (2015) reported the fragmentation of delphinidin derivatives and ternatins obtained from LC-MS^n of *C. ternatea* flower yielded fragment at *m/z* 788 and 773. Moreover, the poor chromatographic resolution of *C. ternatea* flower may indicate that the identified peaks are belongs to the polymeric compounds. Polydispersity of the polymeric compound resulted in poor chromatographic resolution is a challenge in the LC detection and characterization of the compounds (Cheynier, 2012). However, further study on MS^n or NMR should be conducted to confirm the molecular structure of the identified and unidentified peaks.

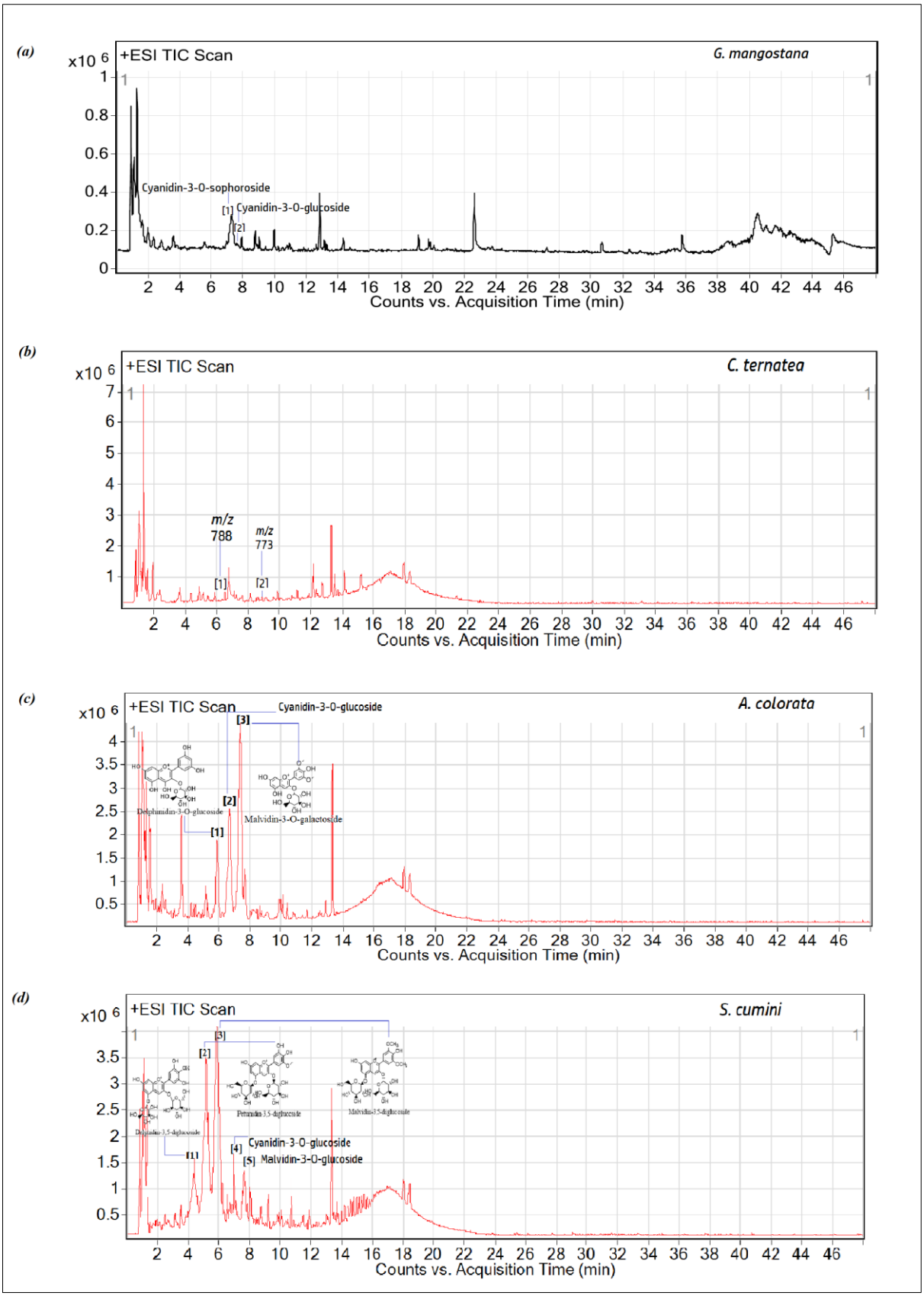

Figure 1. The ESI TICs of LC-MS Q-TOF of the selected pigmented samples

Table 2. Retention time and mass-to-charge (*m/z*) ratio of the identified anthocyanins

Samples	Retention time (min)	Mass-to-Charge (*m/z*) $[M+H]^+$	Description	References
A. colorata fruit	5.89 ± 0.33	465	delphinidin-3-O-glucoside or delphinidin-3-O-galactoside	Cooke *et al.,* (2006); Li *et al.*, (2016); Wang *et al.,* (2016)
	6.85±0.40	449	cyanidin-3-O-glucoside	External standard reference.
	7.68 ± 0.23	493	malvidin-3-O-galactoside	Cooke *et al.,* (2006); Li *et al.,* (2016); Wang *et al.,* (2016)
S. cumini Fruit	4.37 ± 0.60	627	delphinidin 3,5'-diglucoside	Faria *et al.,* (2011); Jampani *et al.,* (2014); Veigas *et al.,* (2007)
	5.86 ± 0.57	641	petunidin 3,5'-diglucoside	
	5.17± 0.56	655	malvidin 3,5'-diglucoside	
C. ternatea flower	6.85 ± 0.59	788	delphinidin derivatives	Nair *et al.,* (2015)
	8.87 ± 0.25	773	delphinidin derivatives	Nair *et al.,* (2015)
	9.87 ± 0.22	303	delphinidin	External standard reference.
	12.13±0.18	287	cyanidin	External standard reference.
G. mangostana peel	7.23 ± 0.49	611	cyanidin 3-sophoroside	Palapol *et al.,* (2009) and Zarena and Sankar (2012) and external standard reference.
	7.6 ± 0.45	449	cyanidin 3-glucoside	

The results of Q-TOF revealed that peak [1],[2] and [3] with $[M+H]^+$ *m/z* 627, 641 and 655 belongs to the delphinidin 3,5'-diglucoside, petunidin 3,5'-diglucoside and malvidin 3.5'-diglucoside, respectively (Figure 1 (*d*) and Table 2). The presence of cyanidin-3-O-glucoside and malvidin 3-glucoside in *S. cumini* were in line with the reports by Jampani *et al.,* (2014) and Veigas *et al.*, (2007). The identified compounds and *m/z* ratio obtained in this study were in accordance with the findings in Faria *et al.,* (2011), Jampani *et al.*, (2014) and Veigas *et al.,* (2007).

Antimicrobial Activity

The antimicrobial activity analysis revealed that *G. mangostana* peel showed the strongest inhibition on the *A. niger*, *B. cereus*, *B. subtilis* and *S. aureus* with minimum inhibitory zone 6.50, 8.50, 6.70, and 7.20 mm, respectively as shown in Table 3. The results obtained were consistent with the previous studies on the inhibition of *G. mangostana* peel against *S. aureus*, *E. coli* and *B. cereus* (Geetha *et al.*, 2011; Mohamed *et al.*, 2014; Palakawong *et al.*, 2013). The *C. ternatea* flower showed minimum inhibition against *B. cereus* at the concentration of 2.5 mg/disc with 8.20 mm of minimum inhibitory zone while no inhibition was observed for the rest of microorganism being tested. The *A. colorata* fruit showed inhibition against *E. coli* and *S. aureus* at the concentration of 2.5 mg/disc with 7.20 and 6.70 mm of inhibitory zone, respectively while *Syzygium cumini* showed inhibition on the *S. aureus* at the concentration of 2.5 mg/disc with 7.20 mm of minimum inhibitory zone. This is consistent with the previous literature on the inhibition of *S. cumini* fruit peel against *S. aureus* (Priya *et al.*, 2013; Singh *et al.*, 2016).

G. mangostana peel showed stronger inhibition against *A. niger*, *B. cereus*, *B. subtilis* and *S. aureus* than other samples, which could be due to the higher concentration of tannins as obtained in phytochemical screening. Tannins have been known to form irreversible complexes with proline-rich protein and resulting in the inhibition of cell wall synthesis (Mamtha *et al.*, 2004). Although tannins are known as a good antimicrobial agent, paradoxically *Candida sp.* are capable of utilizing tannins as a carbon source (Rauha *et al.*, 2000). Therefore, explained the inefficiency of the selected pigmented plant extracts as an antimicrobial agent against *C. albicans* although with the presence of tannins. *E. coli* is resistant to the selected pigmented extract except for *A. colorata*, which could be due to the presence of naringenin or synergistic effect between phenolics presence in the fruit. The presence of

naringenin and other phenolics were previously reported in our previous work in Siti Azima *et al.* (2017). Naringenin and hydrolysed phenolics were found to be effective as an antimicrobial agent against *E. coli* by inhibiting the multiplication of *E. coli* (Rastogi *et al.*, 2008). However, the selected pigmented plant extracts exhibited less potent in antimicrobial activity as compared to the commercial standard antibiotic disc. Most of the phenolic found in plant extract exist in the glycosylated and bounded form, so that did not produce a marked inhibition as compared to the commercial pure antibiotic standard (Rauha *et al.*, 2000).

Table 3. Inhibitory zone of the selected pigmented plants against microorganism tested by disc diffusion method

Samples /Standard	**Conc. (mg/ disc)**	**Diameter of inhibition zone (mm)**							
		Gram positive bacteria			**Gram negative bacteria**			**Fungi**	
		BC	*BS*	*SA*	*EC*	*ST*	*PA*	*CA*	*AN*
G. mangostana peel	0.31	NA	6.70 ± 0.03	7.20 ± 0.03	NA	NA	NA	NA	NA
	0.63	NA	7.70 ± 0.03	8.50 ± 0.05	NA	NA	NA	NA	NA
	1.25	NA	8.00 ± 0.05	9.80 ± 0.03	NA	NA	NA	NA	6.50 ± 0.00
	2.50	8.50 ± 0.05	9.50 ± 0.05	11.00 ± 0.10	NA	NA	NA	NA	7.20 ± 0.03
C. ternatea flower	0.31	NA	NA	NA	NA	NA	NA	NA	NA
	0.63	NA	NA	NA	NA	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA	NA	NA	NA
	2.50	8.20 ± 0.03	NA	NA	NA	NA	NA	NA	NA
A. colorata fruit	0.31	NA	NA	NA	NA	NA	NA	NA	NA
	0.63	NA	NA	NA	NA	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA	NA	NA	NA
	2.50	NA	NA	6.70 ± 0.03	7.20 ± 0.03	NA	NA	NA	NA
S. cumini fruit	0.31	NA	NA	NA	NA	NA	NA	NA	NA
	0.63	NA	NA	NA	NA	NA	NA	NA	NA
	1.25	NA	NA	NA	NA	NA	NA	NA	NA
	2.50	NA	NA	7.20 ±0.03	NA	NA	NA	NA	NA
Chloram-phenicol	0.03	21.00 ± 3.61	22.00 ± 4.58	25.67 ± 2.00	12.67± 1.53	16.67 ± 1.53	NA	NT	NT
Penicillin	0.06	NA	NA	30.33 ± 2.52	NA	NA	NA	NT	NT
Nystatin	0.10	NT	NT	NT	NT	NT	NT	28.00 ± 2.00	19.33 ± 2.52
Water		NA	NA	NA	NA	NA	NA	NA	NA

Notes: No activity is denoted as NA and not tested is denoted as NT. Diameter of inhibition zone was expressed in millimeter (mm) unit. *B. cereus* is denoted by *BC*, *B. subtilis* is denoted by *BS*, *S. aureus* is denoted by *SA*, *E. coli* is denoted by *EC*, *S. typhii* is denoted by *ST*, *P. aeruginosa* is denoted by *PA*, *C. albicans* is denoted by *CA*, and *A. niger* is denoted by *AN*.

Conclusion

Based on the phytochemical screening conducted, *G. mangostana* peel exhibited the strongest indication for the presence of flavonoids, leucoanthocyanidins, quinones, tannins, and anthocyanidin as well as the strongest in diameter of inhibition zone for *A. niger*, *B. cereus, B. subtilis* and *S. aureus*. Three anthocyanins namely delphinidin 3-glucoside, delphinidin 3-galactoside, cyanidin 3-glucoside and malvidin 3-galactoside were for the first time identified in *A. colorata* fruit by using LC-QTOF. This study revealed that phytochemical constituents are related to the antimicrobial activities of the plant samples. In addition, anthocyanins in *C. ternatea* flower, *S. cumini* fruit, and *A. colorata* fruit served as a promising source of natural colourants that can be further applied in food and pharmaceutical industry. The antimicrobial activities of *G. mangostana* peel, *C. ternatea* flower, *A. colorata* fruit, and *S. cumini* fruit may be useful to prolong the shelf-life of food products.

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