Bioactive constituents, antioxidant and antimicrobial activities of three cultivars of wax apple (*Syzygium samarangense* L.) fruits

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Abstract
The present study represents report on bioactive constituents, antioxidant and antimicrobial activities of three cultivars of wax apple fruit. Results revealed that fruits of both Masam manis (pink) and Jambu madu (red) cultivars are the ultimate sources of anthocyanin, carotene and vitamin-C. On the contrary, chlorophyll and titratable acidity content were higher in fruits of Giant green (green) cultivar. The amount of trace elements was high in pink followed by red and green cultivars. The highest amounts of total phenols and flavonoids were in methanol extracts compared to ethanol fruit extracts and these polyphenolic compounds were correlated with antioxidant activities (R^2 = 0.98). In addition, ethanol extracts of leaf, bark and fruit showed good antioxidant activity with IC_{50} values ranging from 283-358 µg mL^{-1} and antimicrobial activity as compared to methanol extracts.

Among the cultivars, red cultivar performed the best antimicrobial activity. Bark and fruit extracts of green cultivar showed the highest antioxidant activity as measured by rabbit erythrocytes haemolysis. LC-MS/MS analysis showed several peaks with fragmentation patterns corresponding to the high antioxidant activity of the water extracted fraction. This current study has shown that wax apple cultivars are ultimate source of bioactive constituents, trace elements and metabolites that have the potential to serve as antioxidant and antibacterial compounds beneficial for human health and may serve as a lead to the development of new pharmaceutical products.

Keywords: Antioxidant, Antimicrobial, Bioactive, Cultivar, Trace element, Wax apple.

Introduction

The wax apple is a nonclimacteric tropical fruit from the Myrtaceae family and botanically identified as *Syzygium samarangense* L.[1]. It is widely cultivated and grown throughout Malaysia and in neighbouring countries such as Thailand, Indonesia and Taiwan. In Malaysia, there are three species which bear edible fruits, namely, the Water apple *(Syzygium aqueum)*, Malay apple (*Syzygium malaccense*) and Wax apple (*Syzygium samarangense*). Wax apple is the most popular of the three in Southeast Asia and produces red, pink and green fleshy fruits. The fruits are eaten raw with salt or cooked as a sauce which has thirst-relieving properties. The fruit is oblong, pear shaped and 5–12 cm in length, with four fleshy calyx lobes and 1- 4 seeds (1-2 cm in diameter).

Myrtaceae plants are mostly used as medicinal plants for the treatment of bronchitis, asthma, diabetes mellitus and inflammation syndromes[2]. In addition, the plants extracts have potent free radical scavenging, antioxidation, antimutation and anticancer activities[3]. The wax apple leaves contain ellagitannins, flavanones, flavonol glycosides, proanthocyanidins, anthocyanidins, triterpenoids, chalcones and volatile terpenoids[4]. The volatile oils from leaves of *Syzygium* species contain a high percentage of terpenoids and γ-terpinene with tannins and related compounds[4]. It has been reported that ethanolic and hexane extracts of these leaves exhibited immunostimulant activity and relax the hyper motility of the gut while the alcoholic extract of the bark showed antibacterial activity[5,6]. The flowers are astringent and are believed to play a role in halting diarrhoea and show antibiotic action against *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Candida albicans*[1].

The fruit pulp of wax apple is a rich source of phenols, flavonoids and several antioxidant compounds and as a result it is believed to have great potential benefits for human health and used in traditional medicine to cure diabetes. Resurreccion-Magno et al[7] reported that wax apple fruit extract showed antihyperglycemic activity in allxan-induced (Type 1 DM) diabetic of mice. The leaves and seeds of wax apple have been shown to have antimicrobial activities against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Cryptococcus neoformans*[8]. Several *Syzygium* species were reported to possess antibacterial, antifungal and anti-inflammatory activities[8,9]. The flavonoids, isolated from wax apple, were reported to possess antihyperglycemic activity, spasmolytic and immunomodulatory activity[7,10,11]. In Malaysia, the powdered dried leaves are used to treat a cracked tongue and a preparation from the root is used to relieve itching and to reduce swelling[1].

In this study, we investigated the bioactive constituents, antioxidants and antibacterial activities of three cultivars of

wax apple and carried out liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis to identify active compounds.

Material and Methods

Experimental site and plant material: The twelve years old wax apple trees of three cultivars were selected from an orchard in Banting, Selangor, Malaysia, located at 2^0 30N, 112^0 30E and $1^0$28 N, 111^0 20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchards is peat with a mean pH of around 4.6. Leaves, barks and fruits of red, pink and green cultivars were collected from the selected plants of this orchard. The experiments consist of three treatments (cultivar) with six replications and a single tree was taken as an experimental unit. The selected uniform branches were tagged properly at the beginning of flower opening until fruit maturation. The experiments were arranged in a completely randomized design (CRD).

Leaf, bark and fruit extracts: After collection of the leaves and barks from the selected plants, the collected leaves and barks were washed in tap water and consequently dewatered and dried at 40°C by using incubator. Dried leaves and barks were ground using an electric blender to produce fine homogenous powder which was then soaked in two selected solvents (95% ethanol and methanol) and preserved in a dark place at room temperature for three days. Samples were filtered through Whatmann No. 1 filter paper. Following filtration, the solutions were evaporated using evaporator at 40 °C and the obtained plant extracts were dissolved in DMSO.

Fractioning: Fractioning was carried out steps as shown in figure 1.

Determination of bioactive constituents and trace elements: The peel colour of the fruits was measured using methods described in Moneruzzaman et al[12] whereas the chlorophyll of fruits was determined at fully ripening stage by methods described by Hendry and Price[13]. The total anthocyanin and carotene contents of the hydrophilic extracts were measured using the pH-differential method with cyanidin-3-glucoside used as a standard as described by Rodriguez-Saona et al[14]. Total ascorbic acid (vit-C) content was determined using the method modified by Hashimoto and Yamafuji[15]. The fruit juice (10 mL) was titrated with 0.1 M NaOH and the results were expressed in terms of percentage citric acid. The percentage of citric acid was calculated by using the formula of Bhattarai and Gautam[16]. Total phenolic contents of various extracts were determined through a protocol based on Folin-Coicalteau method as described by Slinkard and Singleton[17]. Total flavonoid content (TFC) was determined with the aluminium chloride colorimetric assay using catechin as a standard[18].

Determination of antioxidant, antimicrobial activities and LCMS/MS analysis: The erythrocytes haemolysis prevention assay was based on the method described by Rafat et al.[19] According to Haq et al[22], radical scavenging capacity assay was conducted where DPPH (1, 1-diphenyl-2-picrylhydrazil) was used as the free radical by using the following formula: Radical Scavenging Capacity (%) = [(Blank – Sample A) / Blank] x 100. The IC_{50} value for each extract was extrapolated from graphs plotted using the OD value obtained. Paper disc diffusion method of Kil et al[21] was employed to study antibacterial potentials of all the various extracts. The analysis of LCMS/MS of water fraction of wax apple fruits was performed using an Applied Biosystems 3200Q Trap LCMS/MS instrument and detail procedure was carried out according to the method described by Haq et al[22].

Statistical analysis: The data were analyzed by using the MSTAT-C statistical software and a one-way ANOVA was applied to evaluate significant differences in the studied parameters. The means were compared by Duncan's Multiple Range Test (DMRT) and $p = 0.05$ was considered to indicate statistical significance.

Results

Pigments, titratable acidity and Vitamin-C content: The results showed that chlorophyll content was significantly higher in the green cultivar as compared to red and pink cultivars of wax apple (Table 1). Table 1 shows that carotene, anthocyanin and titratable acidity contents were significantly different among the cultivars. The highest carotene and anthocyanin contents were recorded in red and pink cultivars whereas green cultivars contain the lowest. The lowest amount of TA (0.78%) was observed with the red followed by 0.83% in green cultivar. The highest amount of titratable acidity (0.90%) was observed in the pink cultivar (Table 1).

Vitamin-C content varied significantly among the cultivars of wax apple (Table 1). The highest amount of Vit-C content (5.7 mg/100 g) was recorded in pink, followed by red and green cultivars with a Vit-C content of 5.53 and 5.45 mg/100 g respectively (Table 1). Results showed that the amounts of Mn, Zn, Ca, Mg and Cu elements of wax apple cultivars did not vary significantly among them. On the contrary, Fe, Na and K contents of wax apple fruits were significantly higher in pink and red cultivars. The lowest amounts of Fe, Na and K content were recorded in the green cultivars.

Colour development: Peel colour development was significantly variable among the three cultivars. Figure 2 shows that fruit colour development was greatly changed by the fruits of pink cultivar, exhibiting the greatest percentage of peel color from DAA 14 till 35. It was also observed that on 7 days after anthesis the pink and red colour of the fruits of the pink and red cultivars were already started to show, but in green cultivar, the colour

started to show three weeks later. At the 35th day of observation, the pink cultivar fruits showed more or less 99% of pink colour and fruits of the red cultivar showed more or less 95% colour development whereas green cultivar showed only 14% (Figure 2).

Phenols and Flavonoids contents: In this study, all the methanolic extracts produced higher phenolic and flavonoid contents than the ethanolic extracts. The highest phenolic content was observed in methanolic fruit extracts followed by barks and leaves of three cultivars and it was highest in fruits of red cultivar (Table 3).

It was also observed that ethylated bark and leaf extracts of red cultivar exhibited the highest phenolic content compared to pink and green cultivars. Flavonoid content in methylated fruit extracts of red and pink fruits were significantly higher than the green cultivar. Besides, in the case of ethanolic extracts, there is no significant difference among the leaf, bark and fruit extracts of three cultivars.

Antioxidant activity: In this study, antioxidant activities of ethanolic fruits extracts of three cultivars were examined by using DPPH reducing power assays. The IC_{50} values of DPPH assay of these extracts were compared with that of ascorbic acid presented in table 4.

As can be seen from table 4, the highest antioxidant strength was observed in red cultivar followed by pink cultivar while the lowest antioxidant strength was recorded in the green cultivar. We also examined the antioxidant activity of leaf, bark and fruit extracts of three cultivars of wax apple measuring by the rabbit erythrocytes haemolysis (Figure 3).

The negative control (without pre-treatment) obtained a mean of 60% haemolysis whereas ascorbic acid could reduce the haemolysis percentage to 8% in the positive control. All plant extracts (leaf, bark and fruit) of three cultivars of wax apple examined in this study could reduce the erythrocytes haemolysis significantly when compared to negative control. The results also showed that leaf extracts of three cultivars could reduce the erythrocytes significantly than the bark and fruit extracts. Among the three cultivars, green cultivar showed the better effects to reduce the erythrocytes compared to red and pink cultivars.

Antimicrobial activity: The antibacterial activity of wax apple crude extracts was evaluated against four laboratory strains of bacteria including two Gram positive (*Bacillus cereus, Staphylococcus aureus)* and two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) (Table 5). The results showed that all the extracts had the antimicrobial activity but ethanolic extracts showed higher antimicrobial activities than the methanolic extracts (Table 5). Ethanolic bark extracts of red cultivar showed the positive antimicrobial activities against four bacteria and the highest percentage reduction (90%) against *S. aureus*. Pink cultivar showed the antimicrobial activities against *B. cereus* and *E. coli* whereas, green cultivar showed only against *B. aureus.* All the bark extracts of three cultivars exhibited higher antimicrobial activities followed by fruit and leaf extracts. Positive control (30 µg tetracycline, total volume of 50 µL) inhibited the growth of all tested bacteria significantly as compared to the examined extracts (Table 5). The presence of antibacterial activity in different extracts indicated that the extracts possess active compounds which have different activities.

Antimicrobial activity of fractionated fractions: It has been shown earlier that the ethanolic extract of barks of all cultivars was the best extract among all the extracts. The ethanolic extract was then fractionated into polar, semi polar and non-polar fractions. The antimicrobial activities of water and ethyl acetate fractions are presented in table 6. The water and ethyl acetate fractions showed a high activity against all the four bacteria but their antimicrobial activity was not as high as the positive control. For water fraction, the highest inhibition zone (18 cm) was against *S. aureus* and the lowest was against *P. aeruginosa* (10 cm). The highest and lowest inhibition zones for ethyl acetate fractions were 16 and 10 cm against *S. aureus* and *P. aeruginosa* respectively. Water fraction showed higher antimicrobial activity than ethyl acetate fraction but it was lower than the positive control (Table 6).

Correlation between phenols and antioxidant activity: From this study, it was also observed that total phenolic content of methylated fruit extract showed a positive correlation ($R^2 = 0.98$) with antioxidant activity of fruits. Phenolic compounds may show antioxidant activity due to their redox properties which play an important role in absorbing and neutralizing free radical, quenching singlet and triple oxygen or decomposing peroxide.

LC-MS/MS analysis of water extracted fraction: Nominal mass of the active compounds of water fraction of barks of all wax apple cultivars was detected by liquid chromatography tandem mass spectrometries (LC-MS/MS). In this study, we studied the nominal masses of the major actives components in water fraction and observed several peaks at m/z values of 330, 369, 390, 434, 445, 456, 478 and 495 and all the peaks observed were found to have rich fragmentation patterns from the MS/MS spectrum and these patterns correspond to the high antioxidant activity (Figure 4).

Discussion

In this study, we have found that the chlorophyll content was the highest in green cultivar and degradation rate of chlorophyll at colour-turning stage was slow in green cultivar. The anthocyanin and carotene contents of red and pink cultivars were significantly higher compared to green which may be due to slower conversion rate of chlorophyll to carotene or anthocyanin in green cultivar. The variation of pigments content of wax apple cultivars is supported by

the findings of Roussos et al[23] who reported that the anthocyanin and carotene contents vary with cultivar to cultivar and growing conditions. The results showed that titratable acid content was significantly higher in pink compared to red and green cultivars and the range was 0.78- 0.83% citric acid. Our findings were in agreement with the results of Supapvanich et al[24] who reported that range of titratable acidity in wax apple was 0.75% -0.80% citric acid.

It has been reported that Vit-C content in fruits varies among crop cultivars and is affected by environmental factors, harvesting time, vigour and the age of plant. Our results showed that Vit-C content was significantly higher in red and pink cultivars. Similar range of Vit-C in wax apple was observed by Supapvanich et al[24]. The development of red pigmentation in the peels of maturing wax apple fruits is the result of a massive accumulation of anthocyanin content and chlorophyll degradation during the maturation period[25]. Positive values of a* and b* as observed in this work are attributed to carotenoids or anthocyanins present in the skin and were higher in red and pink cultivars. Our previous study reported that at fully ripened stage, jambu madu cultivar contains higher amount of anthocyanin and less of chlorophyll content[26].

Our results showed that cultivars of wax apple were ultimate source of trace elements and minerals. Red and pink cultivars contain plenty amount of trace elements as compared to green cultivars. It was also observed that wax apple cultivars are rich source of phenols and flavonoids contents. In this study, extraction solvents significantly affected the total phenols and flavonoids contents of the prepared extracts. For example, methanolic extracts exhibited better activity compared with ethanolic extracts. These findings were in agreement with previous studies of Cheung et al[27] who reported that methanol can extract the highest amount of phenolic compounds of *Volvariella volvacea* compared to petroleum ether, ethyl acetate and water.

Myrtaceae plants are rich source of biologically active phenols and flavonoid[28]. In this study, it was observed that leaves, barks and fruits of wax apple cultivars are rich source of polyphenolic compounds and the phenols and flavonoids contents were higher in fruits and barks compared to leaves. Our findings were supported by the results of Nonaka et al[29] who reported that wax apple contains abundant phytochemicals like phenols, flavonoids, chalcone and their derivatives. Banerjee et al[30] also reported that *Bruguiera gymnorrhiza* and some other plants contain the higher amount of phenols in barks and fruits compared to the leaves.

Our results showed that bark and fruit extracts showed higher antioxidant activities compared to leaf extracts. Green cultivars showed the best performance than the others. Polyphenolic compounds and tannins performed the antioxidant activities, improved immune function, prevented heart diseases and some cancers[31]. Some studies reported a strong correlation between phenolic content and antioxidant activity in fruits and vegetables[32]. We also found that antioxidant activity of the plant extracts correlated well with the total phenolic content. A positive correlation was observed whereby the antioxidant activity increases when the total phenolic content increases. The study also reported that the extraction solvent for sample preparation has an important effect on the antioxidant activity.

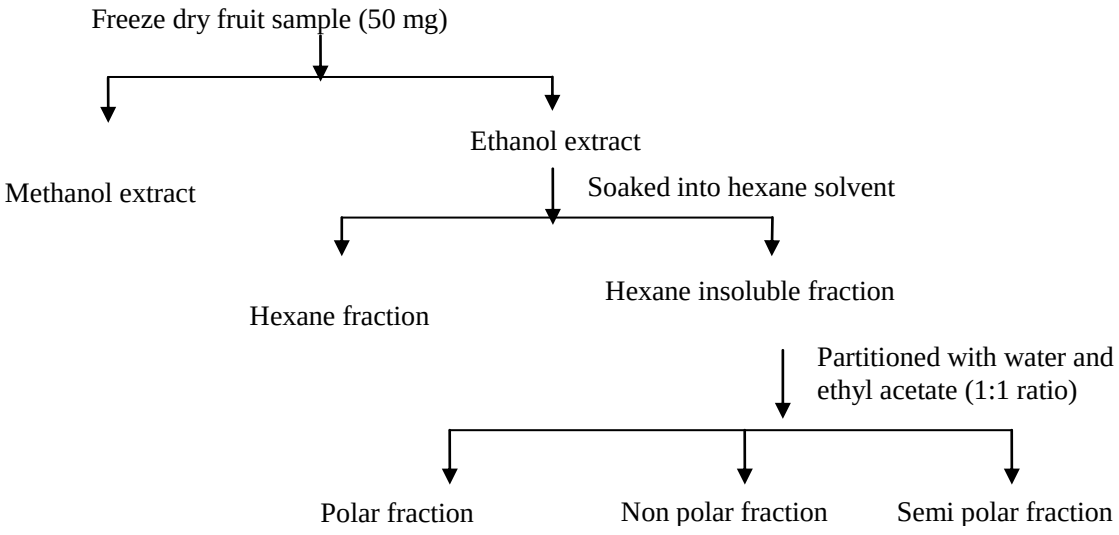


Figure 1: Flow chart for fractionation of active extract of wax apple fruit

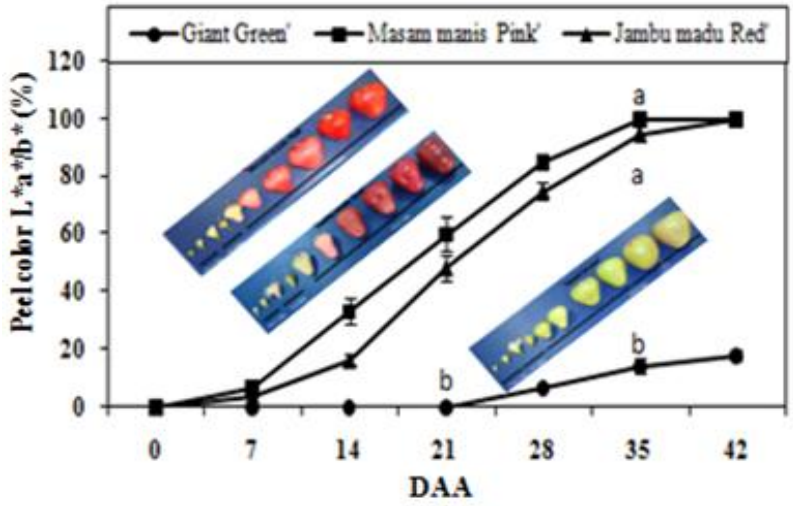


Figure 2: Peel color development of three cultivars of *Syzgium samarangense*. DAA = Day after Anthesis

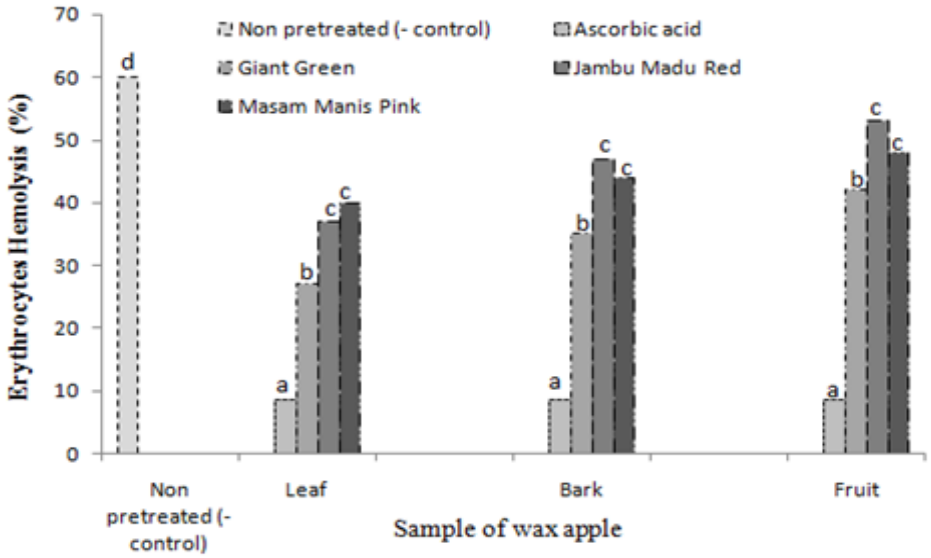


Figure 3: Antioxidant activity of wax apple plant extracts (leaf, bark and fruit) were measured using rabit erythrocytes haemolysis are presented as percentage value. Ascorbic acid (1 mg/1) was used as the positive value.

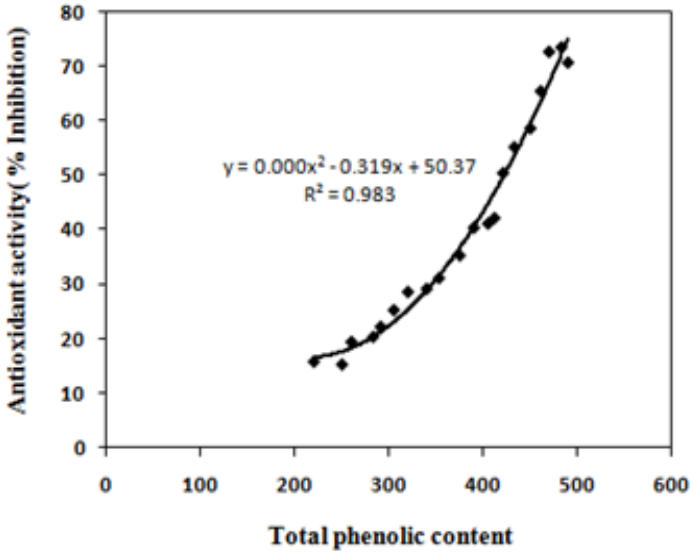


Figure 4: Correlation between the phenolic content and antioxidant activity of wax apple fruits var. Jambu madhu

Several previous study reported that wax apple leaves contain ellagitannins, flavonones, flavonol glycosides, proanthocyanidins, anthocyanidins, triterpenoids, chalcones and volatile terpenoids and these bioactive compounds are known to display inhibitory activities against many microorganisms[4,5,7,29]. These bioactive compounds are the major anti-diabetic and antioxidant components[7]. It has been reported that anthocyanins, flavonoids, phenolic acids and tannins defended against microbial infection[33]. Our study reported that ethanolic extracts of plants leaves, barks and fruits show antimicrobial activity. It was also observed that red cultivar shows higher antimicrobial activity (against four bacteria) than pink and green cultivars. Methylated extract of the leaves and bark has been reported to have antidiabetic, antibacterial, spasmolitic properties and inhibited prolyl endopeptidase[6,7,10]. Houghton[34] reported that secondary metabolites; phenols, tannins, flavonoids and saponins are also capable of destroying or inhibiting the growth of microorganisms.

In this study, ethanolic extracts proved to be the most effective solvent for extracting broad spectrum antimicrobial compounds. Ethanolic extract of barks of wax apple cultivars has been fractionated into three fractions which were water, ethyl acetate and hexane extracted fractions. The amount of hexane-extracted fraction was too little (0.3 g from 50 g of crude extract) and thus was omitted for further bioactivity tests. Both the water and ethyl acetate extracted fractions were used to evaluate the antibacterial activities.

The highest bioactivities were observed in water extracted fraction as compared with ethyl acetate-extracted fraction. Our findings were supported by results of Khokhar and Magnusdottir[35] who reported that water was the best solvent for extraction of tea catechins followed by 80% methanol or 70% ethanol. In this study, LC-MS/MS analysis showed the nominal masses of the major active components in the water fraction and we observed several peaks. All the peaks observed were found to have rich fragmentation patterns and this unique pattern corresponded to the high antioxidant activity of the sample.

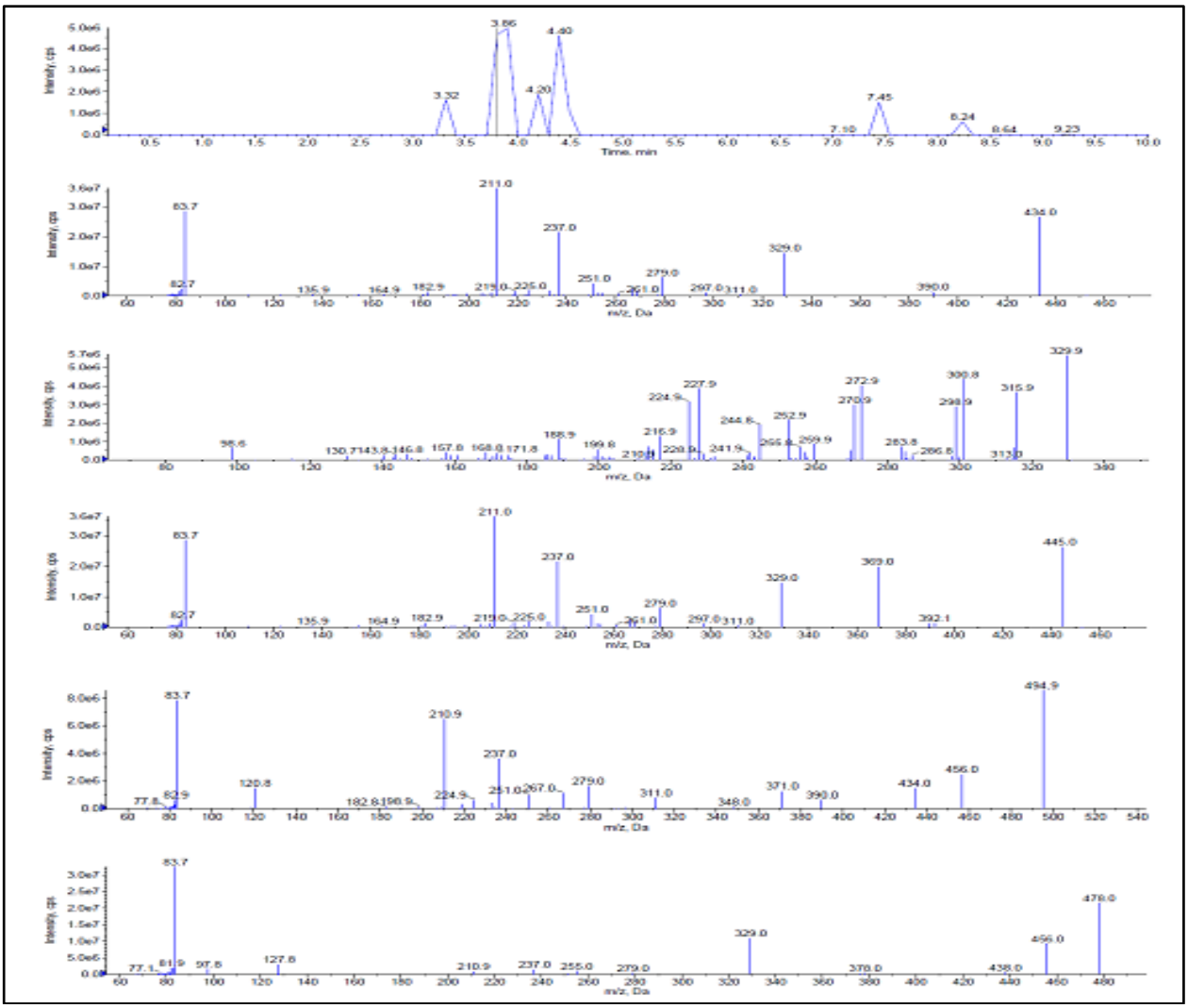


Figure 5: Fragmentation patterns of the major active components in the water fraction from MS/MS spectrum of the major peaks.

Table 1
Content of different pigments in ripened fruit of three cultivars of wax apple

Cultivar	Total chlorophyll (mg L^{-1})	Carotene (µg g^{-1})	Anthocyanin (mg 100 g^{-1})	Titrable acidity (%)	Vit-C (mg 100 g^{-1})
Green	3.43 ± 0.18^{a}	3.83 ± 0.15^{b}	9.5 ± 0.15^{b}	0.83 ± 0.05^{b}	5.5 ± 0.45^{a}
Pink	0.31 ± 0.09^{c}	5.16 ± 0.31^{a}	30.5 ± 0.20^{a}	0.90 ± 0.04^{a}	5.6 ± 0.64^{a}
Red	1.33 ± 0.09^{b}	6.23 ± 0.78^{a}	27.8 ± 0.15^{a}	0.78 ± 0.04^{b}	5.6 ± 0.60a

Means (± S. E) within the same column followed by the same letter, did not differ significantly according to LSD test at ά = 0.01, ns, non-significant * Significant at 0.05 levels, ** Significant at 0.01 levels

Table 2
Content of trace elements in ripened fruit of three cultivars of wax apple

	Fe	Mn	Zn	Na	K	Ca	Mg	Cu
Cultivar	---------- (mg L^{-1})----------							
Green	75 ± 5.0^{b}	2.0 ± 0.20^{a}	2.1 ± 0.23^{a}	38 ± 3.0^{b}	39 ± 3.8^{b}	20 ± 1.8^{a}	15 ± 1.2^{a}	4.3 ± 0.20^{a}
Pink	93 ± 6.9^{a}	1.8 ± 0.35^{a}	2.5 ± 0.28^{a}	45 ± 3.8^{a}	53 ± 3.9^{a}	16 ± 1.5^{a}	22 ± 1.5^{a}	5.2 ± 0.30^{a}
Red	92 ± 6.2^{a}	2.2 ± 0.37^{a}	2.8 ± 0.30^{a}	50 ± 3.5^{a}	55 ± 4.6^{a}	22 ± 1.9^{a}	19 ± 1.4^{a}	5.0 ± 0.24^{a}

Means (± S. E) within the same column followed by the same letter, did not differ significantly according to LSD test at ά=0.01, ns, non-significant * Significant at 0.05 levels, ** Significant at 0.01 levels

Table 3
Total phenol and flavonoid contents of methanolic and ethanolic extracts of leaf, bark and fruits of *Syzygium samarangense* cultivars

Cultivar	Total phenolic content (mg of GAE g^{-1})					
	ML	MB	MF	EL	EB	EF
Green	324 ± 8.23^{b}	480 ± 9.20^{b}	490 ± 8.30^{a}	319 ± 5.34^{b}	430 ± 9.20^{b}	430 ± 8.20^{b}
Pink	359 ± 6.45^{b}	470 ± 8.89^{b}	479 ± 7.80^{a}	345 ± 6.50^{b}	420 ± 8.30^{b}	398 ± 7.36^{b}
Red'	421 ± 7.84^{a}	460 ± 9.25^{b}	465 ± 8.39^{b}	487 ± 7.30^{a}	460 ± 9.31^{a}	447 ± 9.67^{b}
Total flavonoid content (mg CE 100 g^{-1})						
Green	32 ± 2.80^{a}	35 ± 2.30^{a}	45 ± 2.18^{a}	28 ± 1.30^{a}	30 ± 1.30^{a}	26 ± 1.89^{a}
Pink	20 ± 1.25^{b}	34 ± 2.90^{a}	48 ± 2.10^{a}	33 ± 1.28^{a}	36 ± 2.50^{a}	28 ± 1.65^{a}
Red	19 ± 1.45^{b}	37 ± 2.87^{a}	38 ± 1.97^{b}	25 ± 1.30^{a}	29 ± 2.12^{a}	23 ± 1.30^{a}

The data were analyzed by one-way ANOVA and the inhibition means of samples were compared using Duncan's multiple comparison test (DMCT). Mean of different samples labeled with different letters were significantly different in each row ($p < 0.05$).

Table 4
Evaluation of antioxidant activities (% inhibition) of fruit extracts of three cultivars of wax apple

Concentration	Red		Pink		Green		Ascorbic acid
(µg mL^{-1})	% inhibition	IC_{50}	% inhibition	IC_{50}	% inhibition	IC_{50}	IC_{50}
1	15 ± 1.75		15 ± 1.06		10 ± 1.95		
5	19 ± 1.40		17 ± 1.80		13 ± 1.69		
10	22 ± 2.14	283	20 ± 1.09	320	17 ±1.12	358	15.87
50	28 ± 2.53		21 ± 1. 68		19 ± 1.63		
100	31 ± 3.05		29 ± 1.22		24 ± 1.65		
500	73 ± 5.05		68 ±3. 49		64 ± 2.78		

The data were analyzed by one-way ANOVA and the inhibition means of samples were compared using Duncan's multiple range test (DMRT).

Table 5
Antimicrobial activity of methanolic and ethanolic extracts of leaf, bark and fruits of wax apple cultivars

Bacteria	ML	MB	MF	EL	EB	EF	Positive control
Red							**(Tetracy. 30 µg)**
B. cereus	11 ± 0.89^{b}	12 ± 0.85^{b}	14 ± 1.26^{b}	12 ± 0.78^{b}	15 ± 1.35^{a}	15 ± 1.18^{b}	18 ± 1.63^{a}
S. aureus	13 ± 1.69^{b}	17 ± 1.32^{b}	12 ± 0.89^{b}	13 ± 0.53^{b}	18 ± 1.31^{a}	12 ± 0.89^{b}	20 ± 1.95^{a}
E. coli	11 ± 0.78^{b}	13 ± 1.67^{a}	13 ± 0.54^{b}	10 ± 0.93^{b}	14 ± 0.92^{a}	13 ± 0.86^{a}	15 ± 1.58^{a}
P. aeruginosa	$9 \pm .88^{b}$	11 ± 0.76^{a}	10 ± 0.45^{b}	9 ± 0.45^{b}	12 ± 0.85^{a}	11 ± 0.89^{a}	13 ± 1.17^{a}
Pink							
B. cereus	12 ± 1.10^{b}	13 ± 0.80^{b}	13 ± 1.20^{b}	11 ± 0.84^{b}	15 ± 1.97^{a}	15 ± 0.46^{a}	18 ± 1.63^{a}
S. aureus	14 ± 0.89^{b}	17 ± 1.00^{b}	12 ± 0.45^{b}	12 ± 0.98^{b}	18 ± 1.09^{b}	10 ± 0.82^{b}	20 ± 1.95^{a}
E. coli	10 ± 0.78^{b}	12 ± 0.73^{b}	10 ± 0.22^{b}	11 ± 0.71^{b}	13 ± 0.76^{a}	9 ± 0.86^{b}	15 ± 1.58^{a}
P. aeruginosa	10 ± 0.74^{b}	10 ± 0.80^{b}	11 ± 0.21^{b}	9 ± 0.88^{b}	10 ± 0.98^{b}	7 ± 0.69^{b}	13 ± 1.17^{a}
Green							
B. cereus	11 ± 0.75^{b}	13 ± 0.98^{b}	9 ± 0.21^{b}	10 ± 0.88^{b}	13 ± 1.23^{b}	9 ± 0.65^{b}	18 ± 1.63^{a}
S. aureus	11 ± 0.98^{b}	15 ± 1.32^{b}	11 ± 0.90^{b}	12 ± 0.70^{b}	18 ± 1.90^{a}	8 ± 0.51^{b}	20 ± 1.95^{a}
E. coli	10 ± 0.80^{b}	11 ± 1.40^{b}	10 ± 0.75^{b}	9 ± 0.89^{b}	12 ± 1.11^{b}	9 ± 0.80^{b}	15 ± 1.58^{a}
P. aeruginosa	9 ± 0.23^{b}	10 ± 0.93^{b}	8 ± 0.60^{b}	7 ± 0.99^{b}	11 ± 1.56^{b}	7 ± 0.65^{b}	13 ± 1.17^{a}

The data were analyzed by one-way ANOVA and the inhibition means of samples were compared using Duncan's multiple comparison test (DMRT). Mean of different samples labeled with different letters are significantly different in each row ($p < 0.05$).

Table 6
Antimicrobial activity of water and ethyl acetate fractions of fractionated ethanolic extract of *Syzygium samarangense*

Zone of inhibition (cm)			
Bacteria	**Water extract**	**Ethyl acetate extract**	**Positive control**
B. cereus	16 ± 1.57^{b}	12 ± 1.39^{b}	18 ± 1.63^{a}
S. aureus	18 ± 1.62^{b}	16 ± 1.78^{b}	20 ± 1.95^{a}
E. coli	12 ± 0.90^{b}	10 ± 0.85^{b}	15 ± 1.58^{a}
P. aeruginosa	10 ± 0.74^{b}	10 ± 0.79^{b}	13 ± 1.57^{a}
All the data were means of three replicates			

Conclusion

In conclusion, the present studies confirmed the presence of valuable bioactive and antimicrobial constituents and antioxidants in three cultivars of wax apple. The fruits of red and pink cultivars contain the highest amounts of pigments, polyphenolic compounds and antioxidant activities. The crude extracts and fractions of wax apple cultivars exhibited antioxidant properties and antimicrobial activities and red and pink cultivars showed the better antioxidant properties and antimicrobial activities as compared to green cultivar. The ethanolic extract of the bark and water extracted fractions exhibited promising antioxidant properties and antibacterial activities.

Wax apple extracts thus have the potential to be used as bioactive constituents, antioxidant and antibacterial natural sources for the biomedical, healthcare, food and agricultural industries. The LC-MS/MS analysis revealed the nominal mass of the yet unidentified compounds of the extracted water fraction that exhibited high antioxidant activity. Further study needs to be done for identification of these compounds from wax apple cultivars.

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References

1. Morton J. F. and Miami F.L., Java Apple, In Fruits of Warm Climates, 381- 382 **(1987)**

2. Gurib-Fakim A., Medicinal plants: traditions of yesterday and drugs of tomorrow, *Mol. Aspects Med.*, **27(1)**, 1-93 **(2006)**

3. Neergheen V.S., Soobrattee M.A., Bahorun T. and Aruoma O.I, Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activitiesin vitro, *J. Plant Physiol.*,**163(8)**, 787–799 **(2006)**

4. Wong K.C. and Lai F.Y., Volatile constituents from the fruits of four *Syzygium* species grown in Malaysia, *Flavour Fragrant J.*, **11(1)**, 61–66 **(1996)**

5. Srivastava R., Shaw A. K. and Kulshreshtha D. K., Triterpenoids and chalcone from Syzygium samarangense, *Phytochem.*, **38(3)**, 687–689 **(1995)**

6. Ghayur M. N., Gilani A. H., Khan A., Amor E. C., Villasenor I. M. and Choudhary M. I., Presence of calcium antagonist activity explains the use of *Syzygium samarangense* in diarrhoea, *Phytotherapy Res.*, **20(1)**, 49–52 **(2006)**

7. Resurreccion-Magno M. H., Villasenor I. M., Harada N. and Monde K., Antihyperglycaemic flavonoids from *Syzygium samarangense* (Blume) merr. and perry, *Phytotherapy Res.*,**19(3)**, 246 -251 **(2005)**

8. Chandrasekaran M. and Vankatesalu V., Antibacterial and antifungal activities of *Syzygium jambolanum* seeds, *J. Ethnopharmacol.*, **91(1)**, 105 -108 **(2004)**

9. Shafi P. M., Rosamma M. K., Jakil K. and Reddy P. S., Antibacterial activity of *Syzygium cumuni* and *S. travancoricum* leaf essential oils, *Fitoterapia*, **73(5)**, 414-416 **(2002)**

10. Amor E. C., Villasenor I. M., Ghayur M. N., Gilan A. H. and Choudhary M. I., Spasmolytic flavonoids from *Syzygium samarangense* (Blume) Merr. & L. M. Perry, *Z Naturforsch C.*, **60(1-2)**, 67-71 **(2005)**

11. Kuo Y. C., Yang L. M. and Lin L. C., Isolation and inmunomodulatory effects of flavonoids from *Syzygium samarangense, Planta Med.*, **70(12)**, 1237–1239 **(2004)**

12. Khandaker M. M., Boyce A. N. and Osman N., The Influence of Hydrogen Peroxide on the Growth, Development and Quality of Wax Apple (*Syzygium samarangense*, [Blume] Merrill & L.M. Perry var. jambu madu) Fruits, *Plant Physiol. Biochem.*, **53**, 101-110 **(2012)**

13. Hendry G. A. F. and Price A. H., Stress indicators: chlorophylls and carotenoids, In Hendry G. A. F. and Grime J. P., Eds., Methods in Comparative Plant Ecology, Chapman & Hall, London, 148-152 **(1993)**

14. Rodriguez-Saona L. E., Giusti M. M. and Wrolstad R. E., Color and pigment stability of red radish and red fleshed potato anthocyanins in juice model systems, *J. Food Sci.*, **64(3)**, 451-456 **(1999)**

15. Hashimoto S. and Yamafuji K., The determination of diketo-L-gulonic acid, dehydro-L22 ascorbic acid and 1-ascorbic acidin the same tissue extract by 2, 4-dinitrophenol hydrazine 23 method, *J. Biol. Chem.*, **174**, 201–208 **(2006)**

16. Bhattarai D. R. and Gautam D. M., Effect of harvesting method and calcium on post harvest physiology of tomato, *Nepal Agril. Res. J.*, **7**, 37–41 **(2006)**

17. Slinkard K. and Singleton V. L., Total phenol analyses: automation and comparison with manual methods, *Amer. J. Enol. Viticult.*, **28(1)**, 49-55 **(1977)**

18. Zhishen J., Mengchen T. and Jiaming W., The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem.*, **64(4)**, 555-559 **(1999)**

19. Rafat A., Philip K. and Muniandy S., Antioxidant potential and content of phenolic compounds in ethanolic extracts of selected parts of *Andrographis paniculata*, *J. Med. Plants Res*., **4(3)**, 197-202 **(2010)**

]20. MIdadul H., Wirakarnain S., Hossain A. B. M. S., Rosna M. T. and Monneruzzaman K. M., Total phenolic contents, antioxidant and antimicrobial activities of *Bruguiera gymnorrhiza*, *J. Med. Plants Res.*, **5(17)**, 4112-4118 **(2011)**

21. Kil H. Y., Seong E. S., Ghimire B. K., Chung I. M., Kwon S. S., Goh E. J., Heo K., Kim M.J., Lim J. D., Lee D. and Yu C. Y., Antioxidant and antimicrobial activities of crude sorghum extract, *Food Chem.*,**115(4)**, 1234-1239 **(2009)**

22. Haq I., Hossain A. B. M. S., Khandaker M. M., Merican A. F., Faruq G., Boyce A.N. and Azirun M. S., Antioxidant and antibacterial activities of different extracts and fractions of a mangrove plant *Sonneratia alba*, *Int. J. Agril. Biol.*, **16(4)**, 707-714 **(2014)**

23. Roussos P. A., Denaxa N. K. and Damvakaris T., Strawberry fruit quality attributes after application of plant growth stimulating compounds, *Sci. Hort.*, **119(2)**, 138–146 **(2009)**

24. Supapvanich S., Pimsaga J. and Srisujan P., Physicochemical changes in fresh-cut wax apple (*Syzygium samarangenese* [Blume] Merrill & L.M. Perry) during storage, *Food Chem.*, **127(3)**, 912-917 **(2011)**

25. Zhang W., Li X., Zheng J., Wang G., Sun C., Ferguson I. B. and Chen K., Bioactive components and antioxidant capacity of Chinese bayberry (*Myrica rubra* Sieb. and Zucc.) fruit in relation to fruit maturity and postharvest storage, *Eur. Food Res. Technol*., **227(4)**, 1091-1097 **(2008)**

26. Moneruzzaman K. M., Hossain A. B. M. S., Normaniza O. and Boyce A. N., Growth, yield and quality responses to GA3 of wax apple *Syzygium samarangense* var. *Jambu air madu* fruits grown under field conditions, *Afr. J. Biotechnol.*,**10(56)**, 11911-11918 **(2011)**

27. Cheung L. M., Cheung P. C. K. and Vincent E. C. O., Antioxidant activity and total phenolics of edible mushroom extracts, *Food Chem.*, **81(2)**, 249-255 **(2003)**

28. Kahkonen M. P., Hopia A. I. and Heinonen M., Berry phenolics and their antioxidant activity, *J. Agril. Food Chem.*, **49(8)**, 4076–4082 **(2001)**

29. Nonaka G., Aiko Y., Aritake K. and Nishioka I., Tanins and related compounds, CXIX, *Samarangenins* A and B, novel proanthocyanidins with doubly bonded structures, from *Syzygium samarangense* and *S. aqueum*, *Chem. Pharm. Bull.*, **40(10)**, 2671-2673 **(1992)**

30. Banerjee D., Chakrabarti S., Hazra A. K., Banerjee S., Ray J. and Mukherjee B., Antioxidant activity and total phenolics of some mangroves in Sundarbans, *Afr. J. Biotechnol.*, **7(6)**, 805-810 **(2008)**

31. Trease G. E. and Evans M. C., Text-book of Pharmacognosy, 13th Edition, Bailere Tindall, 200-775 **(1989)**

32. Velioglu Y. S., Mazza G., Gao L. and Oomah B. D., Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products, *J. Agril. Food Chem.*, **46**, 4113−4117 **(1998)**

33. Middleton E., Kandaswami Jr. C. and Theoharides T. C., The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer, *Pharmacol. Rev.*, **52(4)**, 673–751 **(2000)**

34. Houghton M., The American Heritage (R. Stedman's Medical Dictionary), Houghton Mifflin Publishers, Boston, USA, 78 **(2002)**

35. Khokhar S. and Magnusdottir S. G., Total phenol, catechin and caffeine contents of teas commonly consumed in the United Kingdom, *J. Agril. Food Chem.*, **50(3)**, 565–570 **(2002)**.

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