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Polyphenolic Compounds and Biological Activities of Leaves and Fruits of *Syzygium samarangense* cv. 'Giant Green' at Three Different Maturities

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Abstract: *Syzygium samarangense* cv. 'Giant Green' is an underutilised fruit that can be found in Malaysia and other Asian countries. Since this fruit is not fully commercialised, the information about its potential health benefits is limited. Thus, this study was carried out to determine the polyphenolic contents (total phenolic and total flavonoid) and biological activities (antioxidant, alpha-glucosidase and antibacterial assay) of 'Giant Green' leaves and fruits at different maturity stages. The young, mature and old leaves, and unripe, half-ripened and ripened fruits were analysed. The results showed that the young leaves increased the TPC and TFC by 35% and 41%, over the old leaves. Similarly, TPC and TFC contents were 37% and 54% higher in unripe fruits compared to the ripened fruits. In addition, young leaves exhibited the strongest scavenging activity towards DPPH, NO and ABTS radicals with IC_{50} values increasing 1.6-fold, 1.7-fold and 2.3-fold, respectively, over the old leaves. However, in fruit samples, only unripe fruits were able to inhibit more than 50% of radicals. A comparable trend was observed in alpha-glucosidase inhibitory assay whereas young leaves and unripe fruits recorded 81% and 99% increases in IC_{50} values, respectively, from young leaves to old leaves and unripe fruits to ripened fruits. Identically, young leaves also showed a significant effect in antibacterial assay with an inhibition zone increase of 19%, 36%, 32%, and 31% in *S. aureus*, *E. faecalis*, *S. typhimurium* and *E. coli*, respectively, over the old leaves. However, only unripe fruits were most effective against all tested bacteria while half-ripened fruits were only effective against *E. faecalis* with a 1.1-fold increase in the inhibition zone compared to unripe fruits. Ripened fruits were resistant to all of the bacteria. These results suggest that the young leaves and unripe fruits of 'Giant Green' cultivar of *S. samarangense* could be a potential candidate for the management of some diseases coming from harmful free radicals or bacterial infection.

Keywords: *Syzygium samarangense*; 'Giant Green' cultivar; phenolic; flavonoid; antioxidant; alpha-glucosidase inhibitory; antibacterial

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1. Introduction

Plant parts such as leaf, flower, fruit, root and stem barks have been used for thousands of years as a traditional medicine which is important in health promotion and wellness. The various plant parts can be used as effective therapeutic agents in reducing the development of certain chronic diseases such as cardiovascular, diabetes, cancer, arthritis,

atherosclerosis, rejuvenation of aged skin and diseases associated with cerebral aging [1,2]. Eleven (11) percent of the 252 basic and essential drugs originated from plant parts while the rest come from synthetic drugs derived from natural precursors [3]. Human bodies are always exposed to harmful reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide (H_2O_2), peroxyl (ROO) radicals, peroxynitrite anion (ONOO) and reactive hydroxyl (OH) radicals which are formed by pollution, smoking or pesticides used in crops [4]. ROS is an essential part of our body at the normal rate in aerobic metabolism and is involved in host defence. Nevertheless, the excess production of ROS is positively correlated to various chronic diseases such as inflammation, diabetes mellitus, cancer, atherosclerosis and hypertension [5,6]. Plant parts are an ultimate source of phenols, flavonoids, tannin and volatile compounds that act as bioactive compounds, natural antioxidants against ROS, reduce oxidative stress and protect humans from chronic diseases [7].

The wax apple (*Syzygium samarangense*) is a non-climacteric tropical fruit that belongs to the genus of *Syzygium* in the Myrtaceae family [8]. The wax apple fruit is also known as jambu air, water apple, wax jambu, bell fruit, makopa, samarang rose apple and java apple [9]. The fruit tree is usually cultivated and grown in Malaysia, Thailand, Taiwan, the Philippines, Vietnam, Laos, China, India, Bangladesh and Indonesia [10]. The fruits are bell or pear-shaped, usually red or light red, pink, green, greenish-white or cream coloured. They are crispy and have an aromatic flavour with a subtle sweet taste. The 'Jambu Madu Red' and 'Masam Manis Pink' are popular cultivars in Malaysia and other Southeast Asian Countries [11,12]. Furthermore, the 'Giant Green' cultivar of wax apple is more aromatic but less popular and underutilised among the cultivars. The cultivar has a spreading canopy with a 3 m canopy width. The density of light green leaves in the canopy of this cultivar is lower than the other two cultivars. The cultivar produces 8 to 10 creamy white flowers in a cluster at the branch tips or in the axils of leaves or any points on the trunk. The flowers bloom within 1 month after bud development and the fruits can be harvested around 50 days after anthesis [8]. The fruit production of this cultivar is non-seasonal and can be harvested three times per year in tropical areas. The fruit length of 'Giant Green' is around 6.3 cm and the diameter is around 5.2 cm [11]. The cultivar has the largest fruit weight (90 g) and produces more seeds than the other two cultivars. The fruit has a rough surface and almost all the fruit is edible. The 'Giant Green' fruits are eaten after ripening, with salt or cooked as a sauce. The leaves of the wax apple contain flavanones, ellagitannins, flavonol glycosides, triterpenoids, anthocyanidins, proanthocyanidins, chalcones and terpenoids [6]. The fruits of the wax apple are a rich source of phenols, flavonoids and several antioxidant compounds and as a result, have potential benefits for human health and are used in traditional medicine.

The plant parts contain valuable chemical content that was believed to benefit human health. In folk medicine, wax apple root is used as a medicine to treat oedema, reduce the itching of the skin and release menstrual cycle pain [8]. The bark and stem of the wax apple have antifungal properties which have been used in wound treatment [6]. The leaves have traditionally been used to treat a cracked tongue, asthma, bronchitis, fever, for bathing purposes, itches and waist pain [13]. The fruit is used to cure mouth ulcers, as a stimulant to increase the urine level, to improve blood circulation in the pelvis and uterus, for treatment of fever, sore throat and to reduce blood pressure. The flower is used to relieve fever and halt diarrhoea [8]. The phenolic and flavonoid contents of wax apple fruits perform antioxidant, antibacterial, antidiabetic and positive cytotoxic activity against the SW-480 human colon cancer cell line [14,15]. The flavonoids, tannins, alkaloids, terpenoids and essential oils of wax apple leaves are also active in anti-inflammatory, spasmolytic, antioxidant, antidiabetic, anticancer and analgesic activities [16,17].

The 'Giant Green' cultivar is underutilised and is not fully commercialised among the three cultivars of wax apple fruits in Malaysia. Previous studies have shown antioxidant and antibacterial activities of this cultivar as well as the presence of valuable phytochemicals such as phenolic, flavonoid, carotenoid and vitamin C in their leaf, fruit

and bark [18,19]. However, studies evaluating the biological activity and the changes of its phytochemical in leaves and fruits of 'Giant Green' at different maturity stages have not been reported in the literature. In addition, the leaves and fruits undergo various morpho-physiological, biochemical and biological changes during maturation and ripening. Hence, the proper maturity stage is crucial for the harvesting of leaves and fruits of the 'Giant Green' cultivar to obtain maximum benefit from them for pharmaceutical uses. Keeping this in consideration, the present study was designed to determine the effects of maturity level on polyphenolic content and biological activities such as antioxidants, alpha-glucosidase inhibitory and antibacterial activities of 'Giant Green' leaves and fruits (i.e., young leaf, mature leaf, old leaf, unripe fruit, half-ripened fruit and ripened fruit). This study proposes that the maturity level of leaves and fruits of the 'Giant Green' cultivar can regulate the accumulation of polyphenolic contents, antioxidant activity, α-glucosidase inhibitory activity and antibacterial activity.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials

The leaves and fruits of the 'Giant Green' cultivar of wax apple were collected several times from the experimental trees of wax apple from an orchard located at Kampung Olek Lempit, Banting, Selangor, Malaysia (1°28 N, 111°20 E), at an elevation of about 45 m above sea level in a hot and humid tropical climate. The type of soil in the orchard was peat and the mean pH was around 4.6 [18]. Three maturity stages of leaves were examined, namely young (YL), mature (ML) and old (OL) leaves whereas for fruits the stages were unripe (UF), half-ripened (HF) and ripened (RF) fruit which were collected and analysed. Five biological replicates of each sample were used in this study. The leaves and fruits were selected based on the physical examination reported by the previous studies. The leaves were collected based on the method reported by Lee et al. [20] (Figure 1). The old leaves were dark green in colour, with a very hard surface. The position of the leaves was lower on the branch, showing the first sign of epiphylly or senescence, with a length of 18–18.5 cm and a diameter of 8.0–8.5 cm. The mature leaves were green in colour, had a hard surface, had a fully developed structure and had a position in the middle of the branch, with a length of 16–16.5 cm and a diameter of 6.0–6.5 cm. The young leaves were light green in colour, had a short growth phase, a soft surface, were fully sized but still lacking in their structure, the position on the branch was higher than the previous stage and the length was 11–11.5 cm and the diameter 3.5–4.0 cm.

The maturity of fruits was selected based on their physical parameters such as colour, texture and size [21] (Figure 1). The ripened fruit was reddish-green in skin colour, greenish-white in pulp, more soft and juicy in texture, had a length of 6.0–7.5 cm and a diameter of 5.5–6.0 cm. The half-ripened fruit was pale green in skin colour, was less soft and juicy in texture, had a length of 5.0–5.5 cm and a diameter 4.5–5.0 cm. Unripe fruit was green in skin colour, was more tough and not juicy in texture, had a length of 3.5–4.0 cm and a diameter of 3.5–4.0 cm.

2.2. Extraction Procedure

The sample was wiped with wet tissue and crushed using a mortar and pestle. The crushed sample (5 g) was soaked in methanol (25 mL) and allowed to stand for three days. Then, it was heated in a water bath at 70 °C for 15 min, followed by being centrifuged at 4000 rpm for 15 min. The supernatant was collected and put in the fumehood to remove all of the methanol solvent. Then, the sample was further lyophilised with the freeze-dried method (24 h). The extract was kept in an air-tight container and stored at 4 °C until analysis.

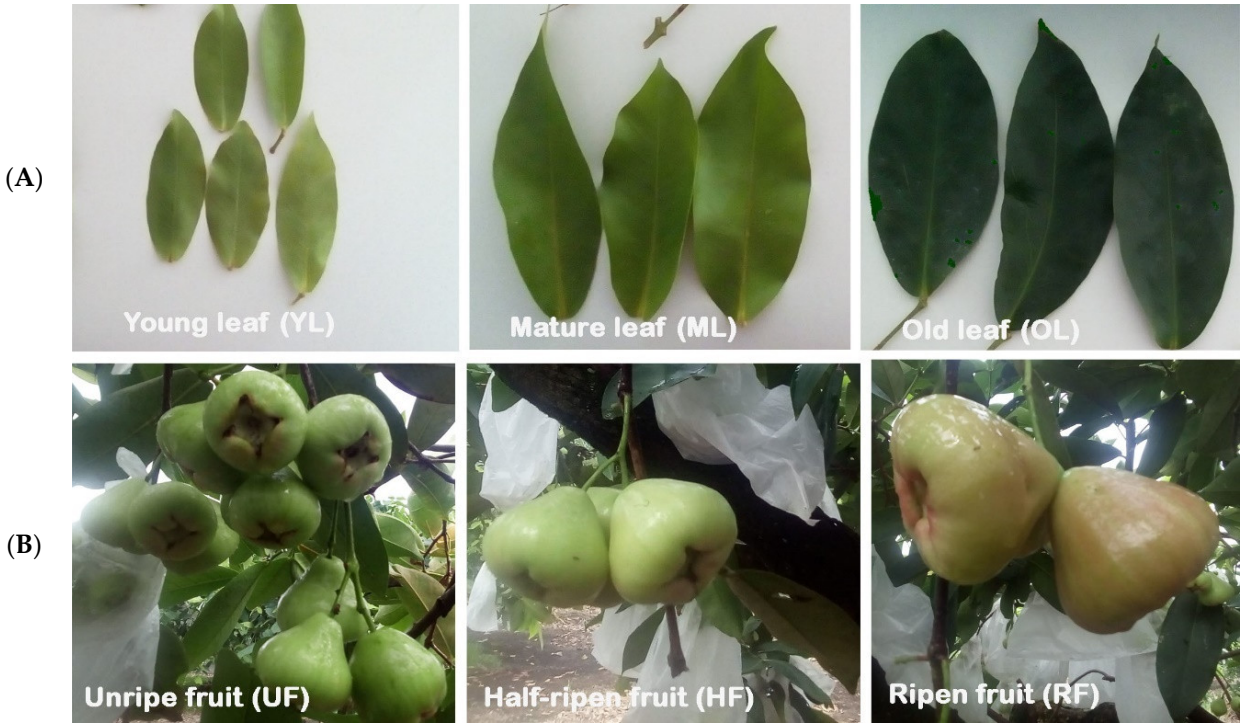

Figure 1. Leaves (**A**) and Fruits (**B**) of 'Giant Green' cultivar of *Syzygium. samarangense* at three maturity stages.

2.3. Percentage of Extraction Yield

The percentage of extraction yield of 'Giant Green' leaves and fruit extracts was calculated according to Zin et al. [22] using the formula below:

$$Percentage\ of\ extraction\ yield = \frac{Weight\ of\ extracts\ (g)}{Weight\ of\ \ raw\ sample\ (g)} \times 100 \quad (1)$$

2.4. Determination of Total Phenolic Assay

The total phenolic content (TPC) assay was measured according to Zin et al. [22]. Approximately 1 mg of standard (gallic acid) and 5 mg of plant crude extracts were dissolved in 1 mL of dimethylsulfoxide (DMSO) which gave 1 mg/mL and 5 mg/mL, respectively, of stock solutions. Then, the standard stock solution was diluted with DMSO by a serial dilution method to produce the final concentrations of 200, 180, 160, 120, 100, 80 and 60 µg/mL. The plant sample was evaluated at a final concentration of 1 mg/mL. So, 60 µL of stock solution from the plant sample was transferred out from the microtube and DMSO was made up to 100 µL. Next, 200 µL of Folin–Ciocalteau was added into standard and plant sample plates, then, the mixture was vortexed vigorously. The reaction was terminated by adding 800 µL of sodium carbonate (7.5%). Then, the mixture was vortexed again and incubated for two hours in the dark at room temperature for blue colour development. The absorbance of the mixture was read at 765 nm using a microplate reader. TPC value of the plant extract was calculated by using the formula below and expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW).

$$C = \frac{cV}{m} \quad (2)$$

where C is total phenolic content, c is the concentration of gallic acid (µg/mL) obtained from the calibration curve, V is the final volume of plant extract and m is the weight of dried extract.

2.5. Determination of Total Flavonoid Assay

The total flavonoid content (TFC) was quantified using a calorimetric assay according to Cunha et al. [23] with some modifications. Quercetin was used as a standard for the calibration curve. Briefly, 0.5 mg of quercetin was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to complete the concentration of stock solution to 1 mg/mL. Exactly 5 mg of plant sample was weighted and diluted in 1 mg/mL of DMSO. The stock solution (quercetin) was prepared by serial dilutions to final concentrations of 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/mL. Then, 140 µL of plant extract (5 mg/mL) was pipetted out into a 96-well plate. Standard quercetin solution and plant extract were separately mixed with 150 µL of aluminium chloride (10%) and 150 µL of potassium acetate (1 M). After mixing, 700 µL was made up with distilled water and the solution was let to stand at room temperature for 30 min in a dark condition. The absorbance was measured at 415 nm wavelength using a microplate reader. The TFC value of the plant samples was obtained from the linear regression equation of quercetin and calculated using the equation below. The results were expressed as milligrams of quercetin equivalents per gram dry weight (mg QE/g DW).

$$C = \frac{cV}{m} \tag{3}$$

where C is total flavonoid content, c is the concentration of quercetin (µg/mL) obtained from the calibration curve, V is the final volume of plant extract and m is the weight of the dried extract.

2.6. Antioxidant Assay

2.6.1. DPPH Radical Scavenging Assay

The antioxidant capacity using 1,1-phenyl-2-picrylhydrazyl (DPPH) radical was determined using the method reported by Zin et al. [22]. Exactly 25 µL of extract with different concentrations (0–250 µg/mL) was added to a 96-well plate and then, mixed with 200 µL of methanolic solution of DPPH (0.1 mM). The control was prepared by substitution of crude extract with DMSO (25 µL) plus DPPH solution (200 µL). The plate was wrapped with aluminium foil and incubated for 30 min in the dark at room temperature. After that, the mixture was measured at 517 nm wavelength using a microplate reader. Quercetin was used as a positive control. For negative control, the equivalent of DMSO without extract was prepared in a similar manner. The percentage inhibition of DPPH radical was calculated as below.

$$Percentage\ inhibition\ (\%) = \frac{Abs.\ C - Abs.\ S}{Abs.\ C} \times 100 \tag{4}$$

where

Abs. C = Absorbance of control

Abs. S = Absorbance of sample in the presence of extract or positive control

2.6.2. Nitric Oxide (NO) Radical Scavenging Assay

Nitric oxide radical scavenging assay was obtained from the reaction of sodium nitroprusside and measured by Griess reagent [24]. Sodium nitroprusside solution (10 mM) was prepared by diluting 0.3 g of sodium nitroprusside into 100 mL of phosphate buffer saline. A volume of 20 µL of extract, as well as a positive control (quercetin) with various concentrations (0–250 µg/mL), was added with 80 µL of sodium nitroprusside solution in a 96-well plate. Then, the plate was incubated under light for 150 min at room temperature. After that, 100 µL of Griess reagent was added to the plate. The Griess reagent was prepared freshly before being used by mixing 1% sulphanilamide solution and 0.1% naphthyl ethylenediamine ethylene diamine dihydrochloride solution in a similar volume. The sulphanilamide solution was prepared by dilution of 0.5 µg of sulphanilamide in 50 µL of 20% glacial acetic acid, and naphthyl ethylene diamine dihydrochloride solution was prepared by dilution of 0.05 µg of naphthyl ethylene diamine dihydrochloride in 50 µL of

distilled water. Then, the plate was kept for 10 min before absorbance of the solution was taken using a microplate reader at 540 nm. The equivalent of DMSO without extract was prepared in the same manner and is known as negative control. The IC_{50} values of extract and positive control were determined. The percentage of inhibition nitrite scavenging was measured using a formula as described in the DPPH assay.

2.6.3. ABTS Radical Scavenging Activities

The ability of the extract to scavenge 2,2,-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) was measured as described by Shalaby and Shanab [25] with small modifications. ABTS salt (0.0768 g) and potassium persulfate (0.0132 g) were dissolved in distilled water (20 mL) to form monocation radical ABTS ($ABTS^+$). $ABTS^+$ is very sensitive to light. Then, the tube containing the $ABTS^+$ solution was wrapped with aluminium foil and allowed to stand in a dark condition for 16 h at room temperature before being used. Then, the solution was further diluted with methanol to an absorbance of 0.700 ± 0.02. Methanolic $ABTS^+$ solution (189 µL) was mixed with 21 µL of various concentrations of all of the extracts and positive control, quercetin (0–250 µg/mL). A similar manner was used to prepare the negative control without extract but substitute with an equal volume of DMSO. Thereafter, the mixture was measured at an absorbance of 734 nm wavelength. $ABTS^+$ radical inhibition was estimated using the equation as described in the DPPH assay.

2.6.4. Reducing Power

The reducing power assay was carried out according to the method of Mayur et al. [26] with slight modifications. Aliquots (20 µL) of plant extract with different concentrations (0–250 µg/mL) was mixed with 30 µL of 0.1 M phosphate buffer (pH 6.6) and 30 µL of potassium ferracyanide (1%, *w/v* in distilled water). The solution was incubated at 50 °C for 20 min. Then, the reaction of the mixture was stopped by the addition of 30 µL of 10% trichloroacetic acid. The solution was mixed well. Next, 20 µL of ferric chloride (0.1% *w/v*) and 70 µL of distilled water were added. The capacity-reducing power of extract was compared to the quercetin as a positive control. The absorbance was recorded at 700 nm by using a microplate reader. The higher absorbance value indicates a higher reducing power of the extract.

2.7. Alpha-Glucosidase Inhibitory Assay

The alpha-glucosidase inhibitory assay was carried out using the method by Misbah et al. [27]. In this assay, the enzyme solution contained 0.2 unit/mL of α-glucosidase enzyme (*Bacillus stearothermophilus*, Sigma Aldrich Brand, Gillingham, UK) and 0.1 M phosphate buffer (pH 7); and the substrate solution consisted of ϱ-Nitrophenyl-α-D-glucopyranoside (0.5 mM) and a similar phosphate buffer was used. For the stock solution, 5 mg of plant extract was dissolved in 1 mL of 98% DMSO. Then, the working solution was prepared by dissolving stock solution with 10% DMSO at certain concentrations (0–45 µg/mL). A total of 10 µL of the extract had been put into a 96-well plate and added with 25 µL of enzyme solution. The mixture was incubated at 37 °C. After 10 min, 25 µL of substrate solution was added and incubated again for 30 min at 37 °C. To terminate the reaction, 100 µL of 0.2 M sodium carbonate was added to the mixture. The absorbance was measured at a 410 nm wavelength. The solution was used as a control in the absence of plant extract, while, without enzyme and substrate it was used as a blank. The percentage inhibition was calculated as in the formula below and the IC_{50} value was determined.

$$Percentage\ inhibition\ (\%) = \frac{(Abs.\ C - Abs.\ CB) - (Abs.\ S - Abs.\ SB)}{1!Abs.C - Abs.CB} \times 100 \quad (5)$$

where,

Abs. C = Absorbance of control (10% DMSO + enzyme + substrate)
Abs. CB = Absorbance of control blank (10% DMSO without enzyme and substrate)
Abs. S = Absorbance of sample (sample solution + enzyme + substrate)

Abs. SB = Absorbance of sample blank (sample solution without enzyme and substrate)

2.8. Antibacterial Assay

The antibacterial activity was evaluated by using agar well diffusion according to the methods of Shanmugam et al. [28]. Two Gram-positive bacteria, (*Staphylococcus aureus* (ATCC 33591) and *Enterococcus faecalis* (ATCC 29212)) and two Gram-negative bacteria (*Escherichia coli* (ATCC 35218) and *Salmonella Typhimurium* (ATCC 14028)), were obtained from the Microbiology Laboratory, Universiti Sultan Zainal Abidin, Besut Campus, Besut, Terengganu. The isolated colony of bacteria was selected with a sterile wire loop and grew in nutrient agar at 37 °C for 24 h. The pure colony was picked and transferred into a Muller–Hilton Broth (MHB) and was incubated at 37 °C until it reached the turbidity of 0.5 McFarland standards (1.5×10^8 CFU/mL). MHA medium was inoculated with 100 µL volume of standardised inoculums of bacterial strains (0.5 McFarland). The bacterial suspension was spread over MHA using a sterile cotton bud and then allowed to stand for a minute to dry the medium. Five of the wells with a 6 mm diameter were made by using a sterile cork borer on the plate. A total of 10 mg of extracts were dissolved in 1 mL DMSO as a stock solution. Using a micropipette, 100, 80, 40 and 20 µL of extracts were added to the respective wells. A total of 100 µL of DMSO was used as a negative control. For a comparative study of antibacterial properties of the wax apple plant, eight commercial standard antibiotics (Gentamycin, Tetracycline, Vancomycin, Amoxycillin, Cephazolin, Neomycin, Metronidazole and Cefaclor) were also analysed. All of the plates were incubated in an upright position at 37 °C for 24 h. The diameter of zone inhibition (mm) was measured. Every experiment was conducted in triplicate.

2.9. Statistical Analysis

Statistical analysis was performed on the data using XLSTAT software version 2014. The significant difference between the parameters studied was analysed by using one-way ANOVA followed by post hoc Tukey's Honestly Significant Difference (HSD) test. Values were expressed as mean ± standard deviation (SD). The confidence levels of all analyses were performed at 95% with $p < 0.05$ representing a significant difference.

3. Results

3.1. Percentage of Extraction Yields

The percentage of extraction yields of leaves and fruits of the 'Giant Green' cultivar of wax apple are shown in Table 1. The results showed that the leaves and fruit extracts yield varied from 3.57 to 4.46% in descending order of old leaves > young leaves > mature leaves. The maturity stages of the leaf did not produce a significant effect on extract yield; however, fruit maturity stages yielded significant effects on the percentage of extract yield. The highest percentage of extraction yield of fruit extracts was recorded in ripened fruit with a value of 4.87%. The lowest percentage of extract yield, 2.98%, was found in half-ripened fruits (Table 1).

3.2. Total Phenolic Content (TPC)

The total phenolic content (TPC) of the 'Giant Green' cultivar of wax apple leaves and fruits was determined to see the effects of maturity stages on the accumulation of phenols in plant parts. The TPC values were calculated using the gallic acid standard curve (Figure 2A). The results indicated that total phenolic content decreased significantly with the advancement of leaf and fruit maturity. The young leaves had the highest TPC followed by mature leaves with a value of 66.56 mg GAE/g DW and 50.37 mg GAE/g DW. The old leaves contained the lowest amount of phenolic content 43.16 mg GAE/g DW. The TPC value of fruit extracts ranged from 34.08 to 54.11 mg GAE/g DW (Table 1). The unripe fruit had a 1.59-fold TPC compared to the ripened fruits.

Table 1. Percentage yield, total phenolic content (TPC) and total flavonoid content (TFC) of leaves and fruit extracts of the 'Giant Green' cultivar of *Syzygium samarangense* at three maturity stages.

Samples	Percentage of Extraction Yield (%)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
Leaves			
Young	3.87 ± 0.03 ^{A}	66.56 ± 0.86 ^{A}	17.25 ± 0.44 ^{A}
Mature	3.57 ± 0.03 ^{A}	50.37 ± 1.05 ^{B}	11.75 ± 0.36 ^{B}
Old	4.46 ± 0.05 ^{A}	43.16 ± 0.83 ^{C}	10.20 ± 0.29 ^{C}
Fruit			
Unripe	3.13 ± 0.02 ^{a}	54.11 ± 0.42 ^{a}	2.34 ± 0.06 ^{a}
Half-ripen	2.97 ± 0.01 ^{a}	39.97 ± 0.31 ^{b}	1.65 ± 0.06 ^{b}
Ripen	4.87 ± 0.02 ^{b}	34.08 ± 0.44 ^{c}	1.07 ± 0.04 ^{c}

Values are expressed as the means ± standard deviation. The different superscript letter refers to significant difference ($p < 0.05$) by comparing the three maturity stage of leaves and fruit samples.

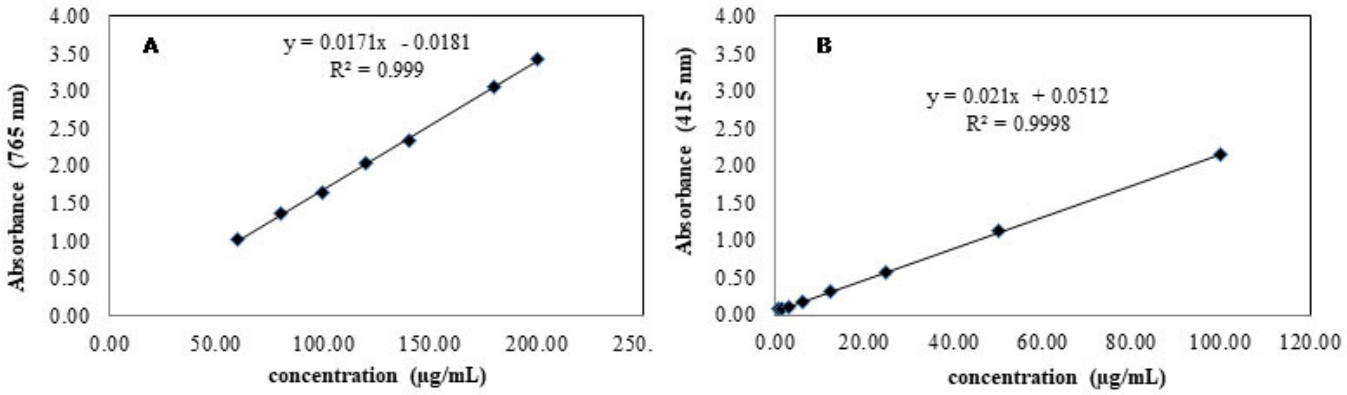

Figure 2. Calibration curve of quercetin and gallic acid standards for determination of total phenolic content (TPC) (**A**) and total flavonoid content (TFC) (**B**).

3.3. Total Flavonoid Content

This result showed that the amount of flavonoid for 'Giant Green' leaves and fruits have a significant effect among three maturity stages (Figure 2B). TFC values of leaf extracts ranged from 10.20 to 17.25 mg QE/g DW with descending order of young leaves > mature leaves > old leaves. The TFC value depicted a 1.7-fold decrease from young leaves to old leaves. For fruit extracts, the flavonoid contents ranged from 1.07 to 2.34 mg QE/g DW (Table 1) in descending order of unripe fruit > half-ripened fruit > ripened fruit. Unripe fruits have flavonoid content two times higher than ripened fruits.

3.4. Antioxidant Assays

3.4.1. DPPH Free Radical Scavenging Activity

Figure 3a illustrates the abilities of 'Giant Green' leaves to scavenge the DPPH radical. The result showed that young leaves exhibited the strongest antioxidant activity, and the weakest was in the old leaves extract. The IC_{50} values of leaf extracts ranged from 13.66 to 21.79 µg/mL (Table 2). Young leaves had 1.6-fold lower IC_{50} values compared to old leaf extract and 1.2-fold lower than mature leaf extract. Meanwhile, the percentage inhibitions of DPPH radicals in fruit extracts are shown in Figure 4a. The unripe fruit showed the strongest antioxidant activity followed by half-ripened while the least one was ripened fruit extract. Between the samples, only unripe fruit showed the ability to scavenge DPPH radicals at more than 50% with IC_{50}: 13.79 µg/mL (Table 2).

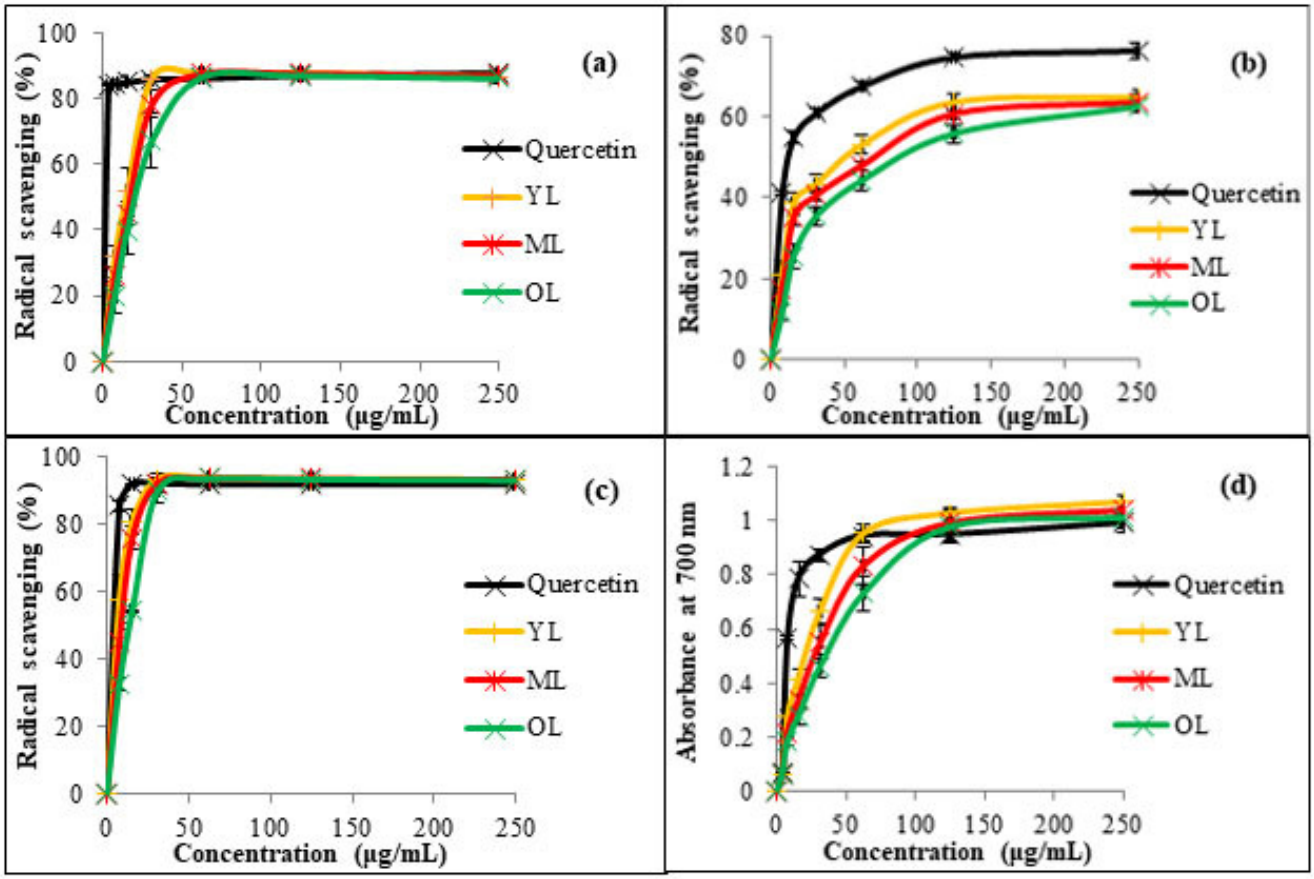

Figure 3. Antioxidant activities of leaves of the 'Giant Green' cultivar of *Syzygium samarangense* at three different maturity stages: (**a**) DPPH; (**b**) NO; (**c**) ABTS; (**d**) Reducing power assay.

Table 2. IC_{50} values of antioxidant assays (DPPH, ABTS and NO) for leaves and fruit extracts of 'Giant Green' cultivar of *Syzygium samarangense* at three maturity stages.

Sample	IC_{50} Value (µg/mL)		
	DPPH	**NO**	**ABTS**
Quercetin	10.20 ± 0.26 ^{Aa}	11.23 ± 0.55 ^{Aa}	4.10 ± 0.10 ^{Aa}
Leaves			
Young	13.66 ± 0.19 ^{B}	51.57 ± 1.71 ^{B}	6.31 ± 0.06 ^{B}
Mature	16.57 ± 0.19 ^{C}	69.29 ± 1.03 ^{C}	8.87 ± 0.31 ^{C}
Old	21.79 ± 0.35 ^{D}	88.13 ± 0.83 ^{D}	14.62 ± 0.43 ^{D}
Fruit			
Unripe	13.79 ± 0.22 ^{b}	87.80 ± 3.50 ^{b}	46.77 ± 0.67 ^{b}
Half-ripened	ND	ND	237.33 ± 1.61 ^{c}
Ripened	ND	ND	ND

ND = Not detected. Values are expressed as means ± standard deviation. The different superscript letter refers to significant difference ($p < 0.05$) by comparing the three maturity stages of leaves and fruit samples. DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid; NO: nitric oxide. Capital letter denotes leaves and small letter fruits.

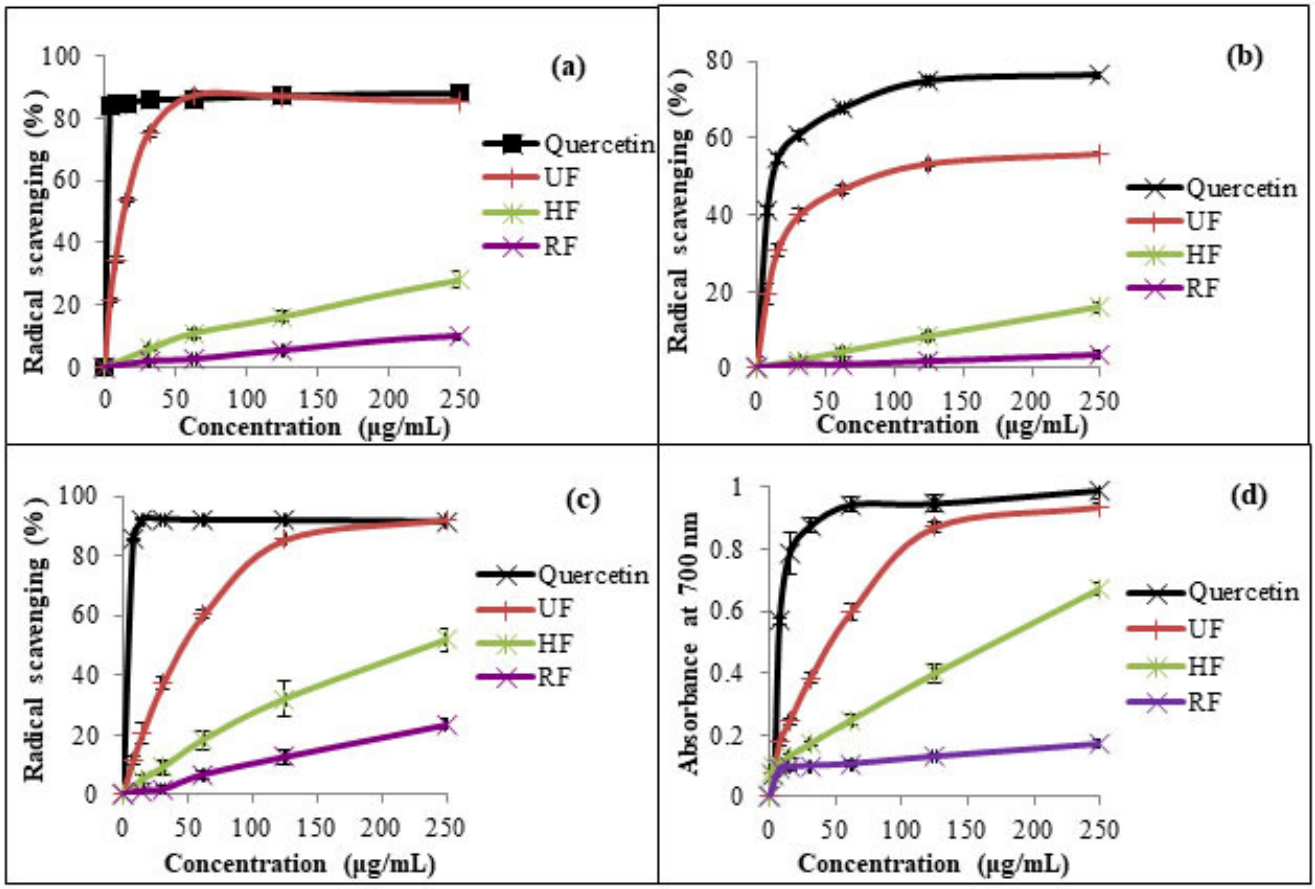

Figure 4. Antioxidant activities of fruits of 'Giant Green' cultivar of *Syzygium samarangense* at three different maturity stages: (**a**) DPPH; (**b**) NO; (**c**) ABTS; (**d**) Reducing power assay.

3.4.2. Nitric Oxide (NO) Radical Scavenging Activity

The capabilities of 'Giant Green' leaf extracts to inhibit the nitric oxide radical is shown in Figure 3b. The results revealed that young leaves had greater NO scavenging capacity followed by mature and old leaves. The IC_{50} values of NO radicals depicted a 41% increase from young leaves to old leaves (Table 2). For the 'Giant Green' fruit, unripe fruit exhibited the strongest scavenging activity towards NO radicals followed by half-ripened and ripened fruit extracts (Figure 4b). The result indicates that only unripe fruit extract had a significant effect to inhibit NO radicals with an IC_{50} value of 87.80 µg/mL (Table 2).

3.4.3. ABTS Radical Scavenging Activity

The percentage inhibition of the ABTS radical scavenging assay of 'Giant Green' leaves is shown in Figure 3c. The results revealed that young leaves had the strongest ability to inhibit ABTS radicals followed by mature leaves and the weakest one was old leaves. The IC_{50} values of leaf extracts ranged from 6.31 to 14.62 µg/mL (Table 2). IC_{50} values recorded a 2.3-fold increase in old leaves compared to young leaves. The abilities of 'Giant Green' fruit extracts in the scavenging of ABTS are shown in Figure 4c in descending order as follows: unripe fruit > half- ripened > ripened fruit. IC_{50} values of fruit extracts depicted an 80% increase from unripe fruit to half-ripened fruit but were not detected in ripened fruit (Table 2).

3.4.4. Reducing Power Activity

The absorbance results of reducing power for 'Giant Green' leaves are shown in Figure 3d. The reducing power of leaf extracts was in a concentration-dependent manner. The reducing power of quercetin, young, mature and old leaves was recorded with values

of 0.99 ± 0.03, 1.07 ± 0.02, 1.03 ± 0.03 and 1.01 ± 0.04, respectively, at a concentration of 250 µg/mL. The young leaves showed higher reducing power followed by mature and old leaves. Furthermore, as seen in Figure 4d, the unripe fruits had a better capability to donate electrons and reduce the ferricyanide complex, while the half-ripened fruits' capability was moderate and the weakest was ripe fruits. The reducing power of all fruit samples also increases with an increase in concentration. The maximum values (at a concentration of 250 µg/mL) of reducing power of unripe, half-ripened and ripened fruits were 0.94 ± 0.01, 0.67 ± 0.02 and 0.17 ± 0.02, respectively. This result indicates that the reducing power in fruit extracts had an 82% decline from unripe fruit to ripened fruits.

3.4.5. Correlation Analysis

Correlation of TPC, TFC and antioxidant assays between leaves and fruits of 'Giant Green' at different maturity stages.

The TP content (Table 3) showed significant positive strong correlations between young leaves with half-ripened fruits, whereas mature leaves with old leaves and ripened fruits and half-ripened fruits with ripened fruits showed significant negative strong correlations. No correlations were detected between young leaves and mature leaves; mature leaves with half-ripened fruits; old leaves with unripe fruits and half-ripened fruits; unripe fruits with half-ripened and ripened fruits and half-ripened with ripened fruits.

Table 3. The correlation coefficients (R value) among leaves and fruits of the 'Giant Green' cultivar of *S. samarangense* in TPC, TFC and antioxidant assays (DPPH, NO and ABTS).

Sample	**Correlation Coefficient of TPC**					
	YL	**ML**	**OL**	**UF**	**HF**	**RF**
YL	1.00					
ML	0.16	1.00				
OL	−0.25	−0.65	1.00			
UF	−0.46	−0.37	−0.14	1.00		
HF	0.64	0.15	−0.22	0.17	1.00	
RF	−0.51	−0.78	0.43	0.28	−0.70	1.00
Sample	**Correlation coefficient of TFC**					
	YL	**ML**	**OL**	**UF**	**HF**	**RF**
YL	1.00					
ML	0.80	1.00				
OL	0.46	0.46	1.00			
UF	0.51	0.21	−0.29	1.00		
HF	−0.30	−0.40	0.00	0.33	1.00	
RF	0.09	0.50	−0.33	0.34	0.05	1.00
Sample	**Correlation coefficient of DPPH Assay**					
	YL	**ML**	**OL**	**UF**	**HF**	**RF**
YL	1.00					
ML	0.92	1.00				
OL	0.92	1.00	1.00			
UF	−1.00	−0.92	−0.91	1.00		
HF	0.09	−0.31	−0.32	−0.10	1.00	
RF	−0.96	−0.78	−0.77	0.97	−0.36	1.00

Table 3. *Cont.*

Sample	Correlation coefficient of NO Assay					
	YL	**ML**	**OL**	**UF**	**HF**	**RF**
YL	1.00					
ML	−0.35	1.00				
OL	0.94	−0.64	1.00			
UF	0.73	−0.90	0.92	1.00		
HF	−0.69	0.92	−0.89	−1.00	1.00	
RF	0.96	−0.60	1.00	0.89	−0.87	1.00
Sample	**Correlation coefficient of ABTS Assay**					
	YL	**ML**	**OL**	**UF**	**HF**	**RF**
YL	1.00					
ML	0.49	1.00				
OL	0.80	0.91	1.00			
UF	−0.98	−0.67	−0.92	1.00		
HF	−0.69	−0.97	−0.99	0.83	1.00	
RF	0.48	1.00	0.91	−0.66	−0.97	1.00

Significant at $p < 0.05$; The value >0.9 a strong correlation, >0.8 a fair strong correlation and >0.6 a moderately strong correlation at p value 0.05. YL: young leaves; ML: mature leaves; OL: old leaves; UF: unripe fruits: HF: half-ripened fruits; RF: ripened fruits; TPC: total phenolic content; TFC: total flavonoid content; DPPH:2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid; NO: nitric oxide.

In the case of TF content (Table 3), only the mature leaves showed significant, positive and very strong correlations with young leaves. Most of the samples showed a moderate and weak correlation with each other. However, no correlation was observed between young leaves and ripened fruits, old leaves with half-ripened fruits and half-ripened fruits with ripened fruits.

Furthermore, in the DPPH assay (Table 3), a significant perfect positive correlation was shown between mature leaves and old leaves, while young leaves with unripe fruits showed a significant perfect negative correlation. Very strong correlations were found between young leaves and mature leaves; old leaves and ripened fruits; mature leaves with unripe fruits; old leaves with unripe fruits; and unripe fruits with ripened fruits. No correlation was shown between young leaves and half-ripened fruits and unripe fruit with half-ripened fruits.

For NO assay (Table 3), there was a significant perfect positive correlation between old leaves and ripened fruits, while a significant perfect negative correlation with unripe fruits and half-ripened fruits was found. In addition, young leaves with old leaves and ripened fruits; mature leaves with unripe fruits and half-ripened fruits; old leaves with unripe fruits and half-ripened fruits; unripe fruits with ripened fruits and lastly, half-ripened fruits with ripened fruits showed very strong correlations.

Moreover, in the ABTS assay (Table 3), only mature leaves with ripened fruits recorded a significant perfect positive correlation. Young leaves with mature leaves and ripened fruits showed a significant positive and weak correlation, while mature leaves with unripe fruits and unripe fruits with ripened fruits showed a significant negative and moderate correlation. However, the rest of the samples correlated very strongly with each other.

Correlation between TPC, TFC and Antioxidant Assays.

The correlations of TPC and TFC with antioxidant assays are as shown in Table 4. There were strong correlations between TPC and antioxidant assays which are ABTS, NO and DPPH in leaves but a moderate correlation in fruit extracts. However, both leaf and fruits extracts were shown to have a strong correlation between TFC and all of the antioxidant assays.

Table 4. The correlation coefficients (R value) of TPC and TFC with antioxidant assay (IC_{50} value) in the 'Giant Green' cultivar of *S. samarangense*.

Variables	Leaves		Fruits	
	TPC	**TFC**	**TPC**	**TFC**
DPPH	0.92	0.85	0.58	0.84
ABTS	0.96	0.91	0.68	0.98
NO	0.90	0.83	0.64	0.92

Significant at $p < 0.05$; TPC: total phenolic content; TFC: total flavonoid content; DPPH:2,2-diphenyl-1-picrylhydrazyl; ABTS:2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid; NO: nitric oxide.

3.5. Alpha-Glucosidase Inhibitory Assay

The percentage of α-glucosidase inhibition of the 'Giant Green' leaves and fruit extracts were plotted as a function of concentration in comparison with standard, quercetin are shown in Figure 5. The results reveal that all of the extracts inhibited α-glucosidase enzyme in vitro. Based on Table 5, young leaves (IC_{50}: 0.80 μg/mL) exhibited good α-glucosidase enzyme compared to quercetin (IC_{50}: 1.54 μg/mL). Mature leaves and old leaf extracts showed appreciable inhibitory activity. The ranking of α-glucosidase inhibition based on the IC_{50} values are as follows: young leaves > mature leaves > old leaves. The IC_{50} values of young leaves depicted a 3.0-fold decrease compared to mature leaves and continuously decreased to 1.8-fold in old leaves as compared with mature leaves. Furthermore, out of the three types of fruit extracts, only unripe fruit and half-ripened fruit extracts showed percentages of more than 50% of alpha-glucosidase inhibitory activity (Figure 5). Unripe fruit exhibited a significant effect of α-glucosidase inhibitory activity followed by half-ripened fruit and ripened fruit. The IC_{50} value dramatically increased to 93% from unripe fruit to half-ripened fruit and continued to increase until it was not detected in ripened fruit (Table 5).

Table 5. IC_{50} values of alpha-glucosidase inhibitory activity for leaves and fruit extracts of the 'Giant Green' cultivar of *Syzygium samarangense* at three maturity stages.

Sample	Alpha-Glucosidase (IC_{50} Value, μg/mL)
Quercetin	1.54 ± 0.75^{ABa}
Leaves	
Young	0.80 ± 0.44^{A}
Mature	2.39 ± 0.06^{B}
Old	4.26 ± 0.37^{C}
Fruit	
Unripe	1.98 ± 0.02^{a}
Half-ripened	28.14 ± 0.34^{b}
Ripened	133.06 ± 3.54^{c}

ND = Not detected. Values are expressed as means ± standard deviation. The different superscript letter refers to significant difference ($p < 0.05$) by comparing the three maturity stages of leaves and fruit samples. Capital letter denotes leaves and small letter for fruits.

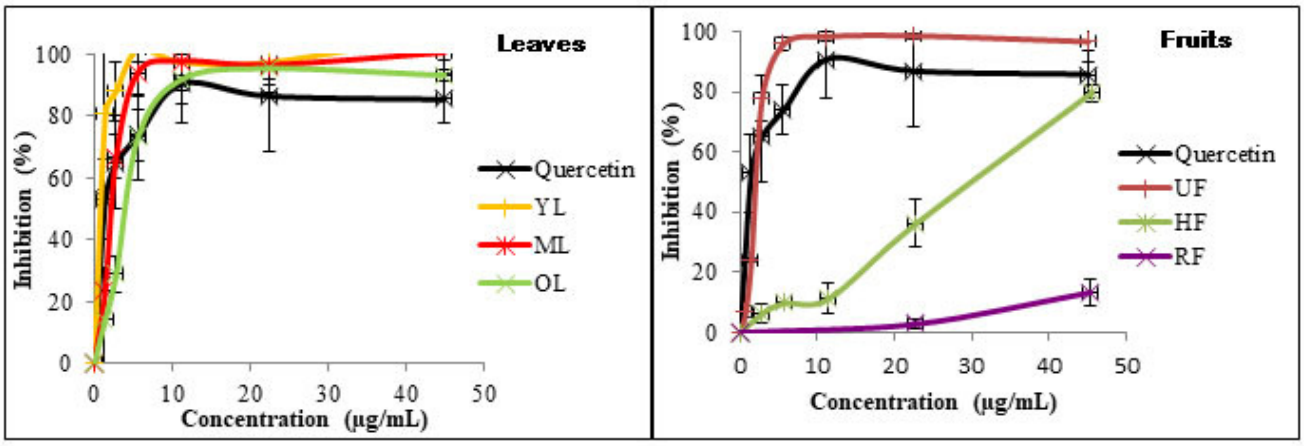

Figure 5. Alpha-glucosidase inhibitory assay of 'Giant Green' cultivar of *Syzygium samarangense* leaves and fruits at three different maturity stages.

Correlation Analysis

Correlation of alpha-glucosidase inhibitory assay between leaves and fruits of 'Giant Green' at different maturity stages.

In the present study, a significant perfect negative correlation was observed between young leaves and mature leaves, then mature leaves with half-ripened fruits (Table 6). Moreover, young leaves with old leaves; unripe fruits and half-ripened fruits; old leaves with half-ripened fruits; and unripe fruits with half-ripened fruits showed a significant positive and very strong correlation, while a significant negative and very strong correlation was recorded between mature leaves, old leaves and unripe fruits; and old leaves with ripened fruits. However, no correlation was found between unripe fruits and ripened fruits.

Table 6. The correlation coefficients (R value) among leaves and fruits of the 'Giant Green' cultivar of *S. samarangense* in TPC, TFC and alpha-glucosidase inhibitory assay.

Sample	Correlation Coefficient of α-Glucosidase Inhibitory Assay					
	YL	ML	OL	UF	HF	RF
YL	1.00					
ML	−1.00	1.00				
OL	0.83	−0.84	1.00			
UF	0.86	−0.85	0.43	1.00		
HF	0.99	−1.00	0.88	0.80	1.00	
RF	−0.52	0.54	−0.91	−0.02	−0.61	1.00

Significant at $p < 0.05$; YL: young leaves; ML: mature leaves; OL: old leaves; UF: unripe fruits: HF: half-ripened fruits; RF: ripened fruits.

Correlation between TPC, TFC and Alpha-Glucosidase Inhibitory Activity

Statistical analysis showed that the leaf extracts have a significant strong correlation between the IC_{50} value of alpha-glucosidase activity and phenolic content with r = 0.938 but is moderate in fruit extracts with r = 0.577. A strong and positive correlation was also observed between alpha-glucosidase inhibitory activity (IC_{50} value) and flavonoid content in leaves and fruit extracts with r = 0.930 and r = 0.850, respectively. The results demonstrated that some phenolic and flavonoid compounds were responsible for the activeness of this activity.

3.6. Antibacterial Assay

3.6.1. Standard Drug Susceptibility/Resistance Testing

Eight standard drugs were investigated to evaluate their capacity for antibacterial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and two Gram-negative bacteria (*Salmonella typhimurium* and *Escherichia coli*). The drugs used in this assay were Gentamycin (CN), Tetracycline (TE), Vancomycin (VA), Amoxycillin/Clavulanic acid 2:1 (AMC), Cephazolin (KZ), Neomycin (N), Metronidazole (MTZ) and Cefaclor (CEC). The results of susceptibility testing are tabulated in Table 7. The data revealed that the overall susceptibility rates in Gentamycin, Tetracycline, Amoxicillin and Neomycin were the highest (100%) against the tested bacteria. Vancomycin was able to inhibit the growth of Gram-negative bacteria (*S. typhimurium* and *E. coli*) only with a susceptibility rate of 50%. Meanwhile, Cephazolin had susceptibility rates of 75% against *E. faecalis* and Cefaclor depicted 50% of susceptibility rates toward Gram-positive bacteria (*S. aureus* and *E. faecalis*).

Table 7. The susceptibility test of standard drug against two Gram-positive and two Gram-negative of bacteria.

Bacterial Strains	Zone of Inhibition (mm)							
	CN (10 µg)	**TE (30 µg)**	**VA (5 µg)**	**AMC (30 µg)**	**KZ (30 µg)**	**N (30 µg)**	**MTZ (5 µg)**	**CEC (30 µg)**
S. aureus	17.05 ± 0.09^{a}	7.07 ± 0.06^{f}	13.07 ± 0.12^{c}	9.10 ± 0.10^{e}	15.07 ± 0.06^{b}	10.10 ± 0.10^{d}	NA	NA
E. faecalis	20.10 ± 0.10^{b}	11.03 ± 0.06^{d}	11.07 ± 0.12^{d}	30.07 ± 0.12^{a}	NA	17.08 ± 0.08^{c}	NA	NA
S. typhimurium	23.13 ± 0.15^{b}	20.10 ± 0.10^{e}	NA	25.10 ± 0.10^{a}	22.20 ± 0.17^{c}	21.10 ± 0.10^{d}	NA	23.08 ± 0.08^{b}
E. coli	21.08 ± 0.07^{a}	18.17 ± 0.15^{c}	NA	15.17 ± 0.15^{f}	17.12 ± 0.10^{d}	19.13 ± 0.12^{b}	NA	16.10 ± 0.10^{e}

NA = not active. Values are the means ± standard deviation for three replicates of experiments (n = 3). Data from same horizontal row with different superscript letter refers to a significant difference ($p < 0.05$). CN: Gentamycin; TE: Tetracycline; VA: Vancomycin; AMC: Amoxycillin/Clavulanic acid 2:1; KZ: Cephazolin; N: Neomycin; MTZ: Metronidazole and CEC: Cefaclor.

3.6.2. Plant Extract Susceptibility/Resistance Testing

The pattern inhibition of Gram-positive (*S. aureus* and *E. faecalis*) and Gram-negative bacteria (*E. coli* and *S. typhimurium*) toward 'Giant Green' leaves and fruits is shown in Figure 6. As seen, the inhibition activity of extracts increases with the increasing concentration. The data depicted that the antibacterial activity against all of the tested bacteria was in a concentration-dose dependent manner. Among the leaf extracts, the young leaves exhibited the maximum inhibition zones in all tested bacteria followed by mature leaves and the minimum inhibition was in old leaves (Table 8). Furthermore, compared to data from the standard drug susceptibility test (Table 7), the young leaf extract displayed very strong activity against all of the bacteria compared to the tested standard drugs. This study suggests that the young leaves have greater potential to be used as medicine in combating certain bacteria. The effectiveness of 'Giant Green' fruits at three maturity stages is recorded in Table 9. The best results are represented by unripe fruit extract which is active against all Gram-positive and Gram-negative bacteria. Half-ripened fruit was only inhibited by one bacterium which is *E. faecalis*. However, the ripened fruit was resistant to all of the tested bacteria. The results indicate that the unripe fruit possessed more powerful antibacterial activity than other mature fruits.

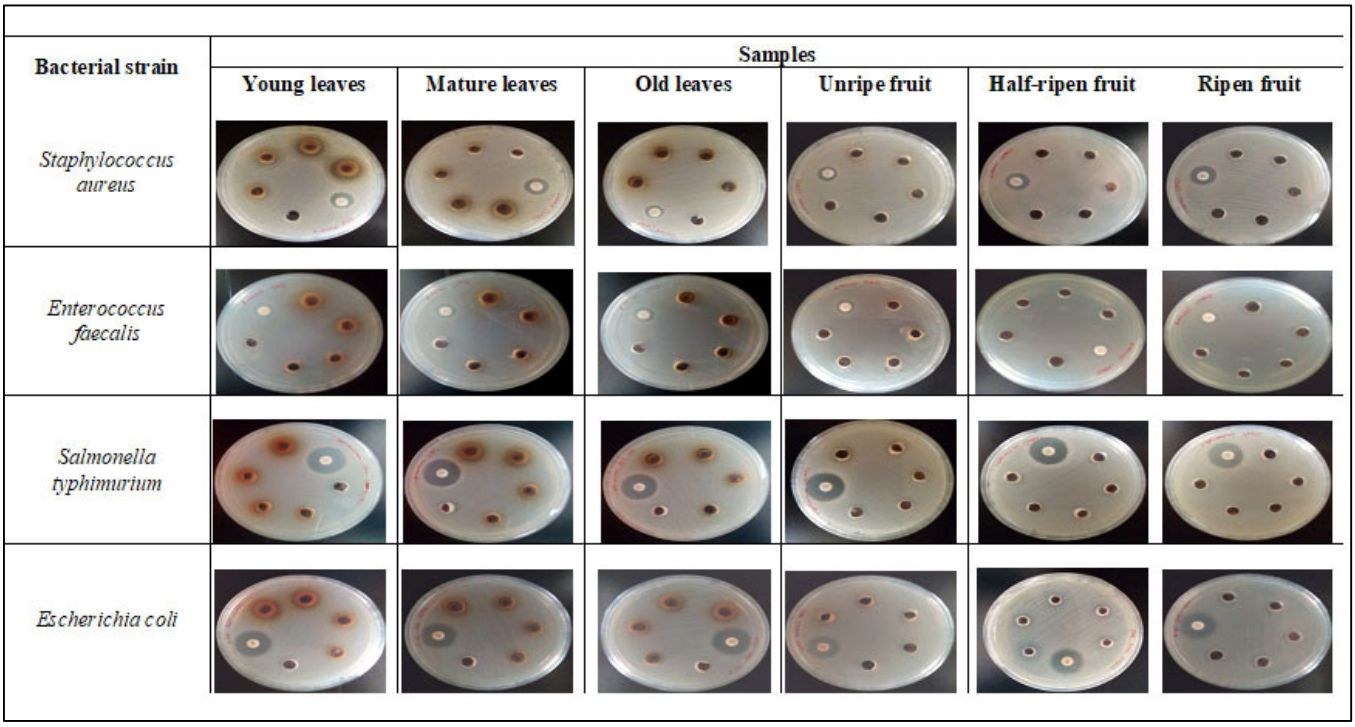

Figure 6. Growth inhibition of some bacteria by methanolic extract of 'Giant Green' leaves and fruits at three maturity stages.

Table 8. The antibacterial activity of the 'Giant Green' cultivar of Syzygium samarangense leaves at three maturity stages.

Leaves Samples	Concentration (μg/mL)	Zone of Inhibition (mm)			
		S. aureus	*E. faecalis*	*S. typhimurium*	*E. coli*
Young	1000	20.47 ± 0.81^{a}	24.13 ± 0.81^{a}	25.40 ± 0.69^{a}	24.33 ± 0.58^{a}
	800	18.47 ± 0.50^{ab}	19.47 ± 0.81^{b}	23.07 ± 1.01^{ab}	22.00 ± 1.00^{ab}
	400	13.80 ± 0.72^{de}	16.13 ± 0.81^{cd}	19.40 ± 1.22^{c}	18.67 ± 0.58^{cde}
	200	10.13 ± 0.81^{g}	13.80 ± 0.35^{def}	15.40 ± 0.69^{def}	17.00 ± 1.00^{def}
Mature	1000	18.33 ± 0.58^{ab}	19.03 ± 0.45^{bc}	20.00 ± 0.50^{bc}	21.13 ± 0.81^{bc}
	800	16.33 ± 0.58^{bc}	16.37 ± 1.42^{cd}	18.00 ± 0.50^{cd}	19.13 ± 0.81^{bcd}
	400	13.33 ± 0.58^{def}	14.70 ± 1.25^{de}	15.00 ± 0.50^{def}	16.13 ± 1.03^{ef}
	200	8.33 ± 6.43^{fg}	13.70 ± 1.25^{def}	10.67 ± 1.61^{g}	15.13 ± 1.03^{fg}
Old	1000	16.67 ± 0.58^{bc}	15.33 ± 0.58^{de}	17.33 ± 0.58^{cde}	16.77 ± 0.68^{def}
	800	15.33 ± 0.58^{cd}	12.67 ± 1.15^{efg}	14.67 ± 0.58^{ef}	15.43 ± 1.25^{f}
	400	12.33 ± 0.58^{efg}	11.67 ± 1.15^{fg}	12.33 ± 1.53^{fg}	12.43 ± 1.50^{gh}
	200	11.00 ± 1.00^{g}	10.67 ± 1.15^{g}	10.67 ± 2.08^{g}	11.77 ± 1.08^{h}

Values are expressed as the means ± standard deviation. Data from the same vertical row with different superscript letters refers to significant difference ($p < 0.05$) by comparing among the three maturity stages of leaf samples.

Table 9. The antibacterial result of the 'Giant Green' cultivar of Syzygium samarangense fruits at three maturity stages.

Fruit Sample	Concentration (μg/mL)	Zone of Inhibition (mm)			
		S. aureus	*E. faecalis*	*S. typhimurium*	*E. coli*
Unripe	1000	13.17 ± 0.29 ^{a}	14.17 ± 0.29 ^{ab}	15.33 ± 0.58 ^{a}	14.33 ± 0.58 ^{a}
	800	11.83 ± 0.76 ^{ab}	13.17 ± 0.29 ^{bc}	13.67 ± 0.58 ^{b}	13.33 ± 0.58 ^{a}
	400	10.83 ± 0.76 ^{bc}	12.50 ± 0.50 ^{bc}	12.67 ± 0.58 ^{bc}	11.67 ± 0.58 ^{b}
	200	9.83 ± 0.76 ^{c}	11.50 ± 0.50 ^{c}	11.67 ± 0.58 ^{c}	10.33 ± 0.58 ^{b}
Half-ripened	1000	NA	15.33 ± 0.58 ^{a}	NA	NA
	800	NA	13.33 ± 0.58 ^{b}	NA	NA
	400	NA	9.67 ± 0.58 ^{d}	NA	NA
	200	NA	8.33 ± 1.15 ^{d}	NA	NA
Ripened	1000	NA	NA	NA	NA
	800	NA	NA	NA	NA
	400	NA	NA	NA	NA
	200	NA	NA	NA	NA

NA = Not active. Values are expressed as the means ± standard deviation. Data from the same vertical row with different superscript letters refers to significant difference ($p < 0.05$) by comparing the three maturity stages of fruit samples.

4. Discussion

Our results indicate that the percentage of extraction yield of leaves and fruits of wax apple extracts varied with the maturity level. The extract yield increased with the maturity stages with the highest percentage of extract yields being recorded in old leaves and ripened fruits of wax apple. The solvent of extraction, pH, temperature, polarity, time of extraction, method of extraction and quantities of sample used can influence the values of the percentage of extraction yield [29]. Singh et al. [30] reported that the morphology of the sample matrix also influences the yield of extraction. The accumulation of extractable compounds depends on the inheritance of the plant, plant developmental stages and responsibilities at different environments. The extracts percentage yield is also affected by the growing condition, light supplied and their genetic variation which modifies the composition of components in plants through their biosynthesis [31]. The highest yield of extracts in old leaves and ripened fruits may be due to increased synthesis and accumulation of phytochemicals at late maturity. The phenolic, flavonoid, steroid, tannin and terpenoid are the phytochemicals that are present in the methanolic extract of leaves and fruits of wax apples [32,33].

Generally, the total phenolic content of all of the 'Giant Green' extracts was in the range of 30–70 mg GAE/g DW. Our results showed that the young leaves and unripe fruit contained the highest phenolic contents compared to mature and old leaves, and half-ripened and ripened fruits. The differences in phenolic between the plant parts may be due to the changes in the type and quantities of polyphenolic compounds during its growth process. Yoshioka et al. [34] revealed that phenolic compounds act as stimulants or inhibitors of enzymes and catalyse as well as control the activities of polyphenol oxidase in plants during the development and ripening stages. In addition, the distribution of phenolic compounds among the plant parts has also often been associated with defence against pathogens or herbivores [35] and function in controlling the biotic and abiotic stress in plants [36]. Similar results were previously reported by Lee et al. [20] with the young leaves of pink cultivar of wax apple containing the higher phenolics such as β-elemene, γ-terpinene and β-caryophyllene compared to the mature and old leaves. Other researchers also found that the unripe fruits of *Sonneratia caseolaris* [37], *Nypha fruticans* Wurmb [38],

Pyracantha [39], *Psidium guajava* [40] and *Malpighia emarginata* DC [41] displayed a high phenolic content compared to ripe fruits.

The results obtained from this study demonstrate that the flavonoid contents of leaves and fruits of 'Giant Green' decreased with the maturity process. The young leaves and unripe fruits recorded the highest flavonoid contents among the other stages. Similar results were reported by Kingne et al. [42] with the young avocado leaves and mango leaves exhibiting the highest flavonoid content compared to mature ones. Young aronia leaves also contained more flavonoid content than the old leaves with a value of 163.7 mg CE/g dry weight and 103.6 mg CE/g dry weight, respectively [43]. On other hand, some studies reported that the flavonoid content of blueberry [44], mangrove apple [39] and papaya [45] increased in the early stages and decreased at the late stages during fruit ripening. The differences in total flavonoid content obtained between the samples in this study and those reported in the previous literature can be influenced by the cultivar and variety, sunlight, growing condition and age of the plants [46].

The present study shows that the young leaves and unripe fruit exhibited the strongest DPPH, NO and ABTS radical scavenging activity as well as the highest reducing power. This may be attributed to higher phenolic and flavonoid compounds in extracts of young leaves and unripe fruits. The plants consisting of more phenolic compounds are believed to contribute to higher antioxidant activity [37]. This finding correlates with previous researchers who reported that the young leaves of *Persea americana* and *Mangifera indica* [42], *Syzygium polyanthum* [47], *Blepharocalyx salicifolius* [48] and *Aronia melanocarpa* [43] exhibited the strongest DPPH activity compared to the old and mature leaf extract. In the same way, Bakar et al. [37] demonstrated that the unripe fruits of *Sonneratica caseolaris* had good scavenging of DPPH and ABTS radical activity for both 80% methanol and aqueous extracts compared to ripe fruit. Taghizadeh et al. [49] revealed that the type of solvent used for the extraction process influences antioxidant activity. Hence, the choice of a suitable polar solvent such as methanol, ethanol or water was crucial to attract the polar compounds such as phenolic and flavonoid in the plant sample. Methanol was reported to be the best solvent to maximise the recovery of the polar compound in the plant extract [36]. This reason may be why the highest phenolic and flavonoid contents were recorded in young leaves and unripe fruit. Furthermore, the electron-donating nature of the substituent groups like -OH, -Cl and -CH_3 in plant compounds are able to inhibit the generation of nitrite and peroxy nitrite anions increasing antioxidant activity [50]. In addition, another factor influencing the variation of radical scavenging activity in the extracts might be influenced by pre- and post-harvest factors [51]. The pre-harvest factors such as the conditions of the environment and agronomic practices could be responsible for the changes in antioxidant levels in the plant parts [52]. The conditions of the environment such as hot or cold temperatures, dry or wet soil, period exposed to sunlight and climatic change cause a decrease or increase in valuable phytochemicals that possess antioxidant value. Zheng and Wang [53] reported that the temperature significantly influenced the antioxidant activity in citrus fruit. Furthermore, the agronomic conditions such as type and quantity of fertilizer, the effectiveness of the irrigation system, maturity stages of the plant and date of sowing influence the antioxidant activity [51]. Previous research by Rajan and Bhat [54] found that the potency of antioxidants in kundang fruit (*Bouea macrophlia* Griffith) is affected by maturity stages in which the unripe kundang fruits possess the strongest antioxidant activity compared to ripe fruit. Furthermore, another factor is the postharvest storage condition [51]. The aspects of time, temperature and light intensities are very crucial to maintain the quality of antioxidant compounds in the plant. Some phenolic compounds such as flavonoid, phenolic acid, anthocyanin and ascorbic acid are sensitive or insensitive to storage temperature, thereby affecting the antioxidant activity [55].

Our study indicates that phenolic and flavonoid compounds in the leaves and fruit of the 'Giant Green' cultivar of *S. samarangense* were correlated to antiradicals. These findings indicate that phenolic and flavonoid are major compounds contributing to antioxidant activity, especially in the scavenging of harmful radicals generated from oxidative stress.

Ng et al. [56] found a correlation between TPC and TFC with DPPH and ABTS radical scavenging activity of selected medicinal plants. Furthermore, Majumder et al. [57] also revealed a good correlation between TPC and TFC with in vitro and in vivo antioxidant activities, which indicates that phenolic compounds possess strong antioxidant capacities in *S. samarangense* leaves. Zielinski et al. [58] stated that the chemical structure of the phenolic compound may influence the variation of antioxidant capacity. The compound with the highest ability to delocalise the lone electron around the aromatic ring possesses stronger antioxidant activity. However, a decrease in hydroxy and methoxy substituents and the increase in the electron-withdrawing group in the aromatic ring reduces the radical scavenging activity. Another factor contributing to the relationship of phenolic and flavonoid with antioxidant activity was the ability of these compounds to inhibit the oxidant enzyme such as nitric oxide synthase (NOS), xanthine oxidase (XO) and NADPH oxidase (NOX). Research reported by Nakao et al. [59] found that hesperetin can inhibit the production of XO, which decreases the formation of free radicals. Furthermore, phenolic compounds that directly react with reactive oxygen species (ROS) or reactive nitrogen species (RNS) also enhance antioxidant capacity. These compounds can act as a safeguard to control the accumulation of ROS and RNS in the body. In addition, the synergism among the phenolic compound or with other groups also influences the efficiency of antioxidant activity [60]. For example, the interaction of phenolic compounds with ascorbic acid and vitamin E [61], flavonoids with protein [62] and phenolic with phenolic such as naringenin with hesperidin [63] significantly increases the antioxidant capacity.

One of the properties of the wax apple plant is to have antidiabetic properties. This was proven in our study when the 'Giant Green' cultivar of *S. samarangense* showed a significant effect in alpha-glucosidase inhibitory activity, especially in young leaves and unripe fruit extracts. Phenolic and flavonoid compounds have been reported to have a major effect on alpha-glucosidase inhibitory activity [64]. Nurnaeimah et al. [65] reported that the high α-glucosidase inhibitory activities represent a potential antidiabetic agent. The highest quantity of phenolic and flavonoid compounds in young leaves and unripe fruit extracts could be the cause of why both extracts showed the strongest alpha-glucosidase inhibitory activity than other maturity stages. Some of the literature reported that the antidiabetic activity in *S. samarangense* plant parts was positively correlated with phenolic contents [64–67]. Hu et al. [68] also found that resorcinol derivatives in the *S. samarangense* leaves could inhibit the strongest alpha-glucosidase activity. Fatanah et al. [69] indicated that the youngest plants need more phenolic compounds that are believed to function in defending against ultraviolet radiation and aggression by pathogens compared to old plants. This might be one of the reasons why an active defensive mechanism by secondary metabolites in young plants exhibited significant antidiabetic activity more than in old plants. Furthermore, the hypoglycaemic effect in plant extracts also increases the efficiency of alpha-glucosidase inhibitory activity. Phenolic and flavonoid compounds have been identified to possess a hypoglycaemic effect in reducing blood glucose levels [70].

From this analysis, it is clear that the phenolic and flavonoid content of leaves and fruits of the 'Giant Green' cultivar of *S. samarangense* were correlated with alpha-glucosidase inhibitory activity. The inhibition capacity of alpha-glucosidase is closely related to the chemical structures of phenolic and flavonoid compounds such as the position and quantity of the hydrogen group attached to the aromatic ring, the methyl group substituent and the complexity of the structure [71]. The more OH group attached at the aromatic ring, the more effective the compound to inhibit alpha-glucosidase activity [72]. Then, the flavonoid compound with glucose moiety at the C-3 position and the methyl group at the position C-7 are more effective toward α-glucosidase inhibitory activity [73]. The addition of hydrophobic and bulky substituents such as methyl substituent in compounds decreases the activity of alpha-glucosidase. The synergistic effect also exhibits the greatest inhibitory activity of alpha-glucosidase [74]. The combination of phenolic compounds with other compounds such as glyceolin and luteolin significantly improved the alpha-glucosidase inhibitory activity [75].

The study found that young leaves and unripe fruit of the 'Giant Green' cultivar of *S. samarangense* possess the strongest antibacterial activity of the maturity stages. Both of the extracts also were considered the most promising for their activity against both Gram-negative bacteria, *E. coli* and *S. typhimurium* where it is more difficult to find the compounds that have the capability to penetrate the double membrane surrounding the bacterial cell wall [76]. Small hydrophilic molecules which are lipophilic macromolecules have properties to pass through the outer membrane of Gram-negative bacteria [77]. This study revealed that young leaves and unripe fruits consist of valuable chemical compounds that have the strongest ability to penetrate both the inner and outer membrane. These compounds might be accumulated in the plasma membrane resulting in the loss of cellular constituents, changes in cellular structure and function and disturbed metabolism [78]. Other than that, it is also capable of inhibiting the synthesis of the bacterial cell wall, causing the depletion of energy, mutation, cell damage and lastly leading to death [79,80]. Chemical compounds such as flavonoid, triterpenes, sterol, tannin, terpenoid and alkaloid were believed to significantly contribute to antibacterial activity [81,82]. Research by Khandaker et al. [19] showed that phenolic and flavonoid content in wax apple leaves, bark and fruit extracts are able to inhibit the growth of four bacteria, *Bacillus cereus*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. Moreover, the synergistic effect of the different chemical constituents, even present in small quantities, also influences the effectiveness of the extract to inhibit the growth of certain bacteria [83]. Future studies of young leaves and unripe fruit should explore the lead compound that gives greater potency in antibacterial activity, especially against Gram-negative bacteria.

5. Conclusions

This study has shown that young leaves and unripe fruit of 'Green Giant' cultivar of *Syzygium samarangense* had the highest total phenolic content, total flavonoid content and had strongest antioxidant, alpha-glucosidase inhibitory and antibacterial activities compared to other maturity stages. It can be concluded that young leaves and unripe fruits of the 'Giant Green' cultivar are good sources of natural antioxidants that can be used to scavenge harmful free radicals. Furthermore, they can also be used in food preservatives or the pharmaceutical industry. However, further study needs to be conducted for the identification of bioactive compounds that serve as effective antioxidant, antidiabetic and antibacterial agents in these samples.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

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