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# Total Phenolic Contents, Antioxidant Activities and Organic Acids Composition of Three Selected Fruit Extracts at Different Maturity Stages

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## Abstract

The total phenolic contents (TPC), antioxidant activities and organic acid composition of *Salacca zalacca*, *Mangifera indica* L. Chok Anan and *Baccaurea motleyana* Hook. F. extracts at different maturity stages were evaluated by using modified Folin-Ciocalteu assay at 765 nm absorbance, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and HPLC RP18 column at 215 nm. TPC content ranged from 63.9 mg GAE/100g to 381.23 mg GAE/100g, whereas the antioxidant activity ranged from 3.10% - 84.45%. The *M. indica* L. Chok Anan contained malic acid and citric acid in the range of 43.897-73.396 mg/100g FW and 2.681-18.428 mg/100g FW at young to ripe stages, respectively. *B. motleyana* Hook. F. contained citric acid and tartaric acid in the range of 4.649-6.114 mg/100g FW and 3.645-4.365 mg/100g FW at young to ripe stages, respectively. *S. zalacca* contained malic acid and citric acid in the range of 4.270-17.926mg/100g FW and 0.845-3.284 mg/100g FW at young to ripe stages, respectively. The highest TPC and antioxidant activity were found in the young stage of *S. zalacca* fruit extract with lowest inhibitory concentration (IC50) valued at 0.57 mgml-1. There was a positive linear correlation between the TPC and antioxidant activities of the fruit extracts. Malic acid is the predominant organic acid in *M. indica* L. Chok Anan and *S. zalacca*, while citric acid is the predominant organic acid in *B. motleyana* Hook. F. This research suggests that the studied fruits at young stage are a better source of TPC and antioxidants. The tested fruits showed a decrease in the concentration of organic acids with ripening.



## 1. Introduction

Malaysia is enriched with variety of tropical fruits. Some of the fruits are seasonal while the other comes in all year round. When fruit seasons are nearby, fruits in Malaysia will be such in demand and we could easily find plenty of tropical local fruits selling at rural roadside stalls, night market and many other locations around the country. These fruits are also rich with essential nutrients and vitamins; therefore it is actually good for human consumption. Although the tropical fruits in Malaysia are high in demand, there are still plenty of underutilized local fruits in Malaysia, this include *Salacca zalacca*, *Mangifera indica* L. Chok Anan and *Baccaurea motleyana* Hook. F.

Phenolic compounds or phenols are substances found on plants, including various kinds of fruits and vegetables. Phenolic compounds are

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essential for the sensory quality of fruits, such as color, astringency, bitterness and flavour (Herrmann, 1990). Some of these compounds are known as natural antioxidants, while these antioxidants are believed to have beneficial effects on maintaining human health, associated with heart diseases and cancer (Newmark, 1996; Velioglu *et al*., 1998). According to Ikram *et al.*, (2009), there are great variations in the antioxidant capacity and total phenolic compound of the underutilized fruits. The correlation results indicated that along with phenolic compounds, other antioxidant components such as vitamins C and E and carotenoids could also contribute to the antioxidant capacity of underutilized fruits.

Organic acids are great significance in plants. As intermediates in the metabolic processes of the fruits including respiration and photosynthesis, these acids are contributed in growth, maturation and senescence. Higher content of organic acids make fruit juices having a relatively low pH level. Some of the major acids in fruits include citric, malic and tartaric acids (Karadeniz, 2004). The changes in composition of organic acids during ripening process are important to flavour development and can affect the chemical and sensory characteristics of fruits (Abeles & Takeda, 1990).

Previous work done mostly concentrated on the relationship between antioxidant activity and total phenolic compound in fruit. However, no research was done to study the correlation between phenolic content, antioxidant activity and composition of organic acid in local fruits of Malaysia with different maturity stages. Thus, the aim of this study was to determine the total phenolic compound, antioxidant activity and composition of organic acids in *Baccaurea motleyana* Hook. F, *Magnifera indica* L. Chok Anan and *Salacca zalacca* extracts at different maturity stages to further understand the relationship between them with regards to maturity of these underutilized fruits.

## 2. Methodology

### 2.1 Sample preparation

This study covers three local fruits (*Salacca zalacca*, *Mangifera indica* L.. Chok Anan and *Baccaurea motleyana*) which are commercially available at a local stall along the Machang- Tanah Merah and Jeli road in Kg Gemang, Jeli, Kelantan. Selected fruits with young, mature and ripe stage were purchased from stalls owner who claimed the fruits come from local sources. The ripeness of the fruit was judged by observing the changes in size, colour, texture and flavour, which make the fruit ready for consumption (Moing *et al.*, 1998). After removing the non-edible parts, edible fruits was cut into small pieces and grounded into slurry by using a food blender. For extraction, 1 g of sample was weighted and homogenized with 10 ml of 80 % aqueous methanol for 3 days. The extract was ultra-centrifugated for 10 minutes at 12,000 rpm. The supernatant was used for the evaluation of total phenolic content, antioxidant activity and organic acids composition.

### 2.2 Determination of Total Phenolic Content

Total phenolic content of the extract was evaluated by using modified Folin-Ciocalteu assay (Singleton & Rossi, 1965). For the test, 20 µl of sample extract was mixed with 100 µl of 10% Folin-Ciocalteu phenol reagent before 1.58 ml of distilled water was added into a test tube. The reaction was left for 5 min before 300 µl of 7.5% sodium carbonate was added. The mixture was incubated in dark for 1 hour at room temperature to allow colour development. The absorbance was recorded at 517 nm using spectrophotometer (Thermo Scientific, Model Genesys 20). Calibration curves were constructed for the solvents with gallic acid (GAE) concentration in range of 0-500 $mgL^{-1}$. Total phenolic content was expressed in mg Gallic acid equivalents (GAE)/100g. All samples were analyzed in 10 composite replications.

### 2.3 Determination of Antioxidant Activity

The determination of antioxidant activity was conducted by DPPH radical scavenging assay with slight modification (Musa *et al.*, 2010). Stock solution containing 0.004 % DPPH was prepared by dissolving 0.012 g of DPPH into 300 ml of methanol. The stock solution was kept in 4 °C for further used.

Briefly, the sample extracts was diluted through a serial dilution into a concentration of 0.625,

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1.25, 2.5, 5 and 10 mgml$^{-1}$. For the reaction, 1 ml of diluted solution were then added with 1 ml of prepared DPPH solution, and was incubated in dark for 30 minutes at room temperature. The absorbance was recorded at 517 nm using spectrophotometer (Thermo Scientific, Model Genesys 20). All samples were analyzed in ten composite replications and results were presented as mean values ± standard deviation. Percentage of DPPH scavenging activity (%) was calculated as equation (1) below, whereas the IC50 values were evaluated from DPPH scavenging activity (%) vs concentration plot, using regression equation.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{“A” blank- “A” sample}}{\text{“A” blank}} \times 100\% \quad .... \quad (1)$$

Where “A” is the absorbance in 517 nm

## 2.4 Extraction of Organic Acids from fruit

For sample preparation, 20 g of flesh from the fruits (skin and seed were removed) was sliced and homogenized with 10 ml of distilled water using blender. The purees were clarified by centrifugation at 12,000 rpm for 15 minutes. The supernatant was filtered through a 0.25 μm Millipore filter. Sample were kept in a refrigerator and analyzed within 12 hours of preparation.

## 2.5 Analysis of organic acids by using HPLC

For HPLC separation, the SHIMADZU SPD M20A Prominence Diode UV absorbance detector was set at 215 nm. Chromatographic separation was performed on a Thermo Scientific, Hypersil Gold Pim (250 x 4.6 mm) RP18 column. The mobile phase consisted of 5 % methanol in 0.025 M potassium dihydrogen phosphate (pH was adjusted to 2.20 by dilute phosphoric acid) was delivered at flow rate of 1.0 mlmin$^{-1}$. The mobile phase was filtered through Nylon membrane filter (0.45 mm) and degassed by sonication before use. To obtain the calibration curves, 2 mgml$^{-1}$ mixtures of standards at 50 mgml$^{-1}$, 250 mgml$^{-1}$ , 750 mgml$^{-1}$ and aqueous solution containing 1 mgml$^{-1}$ of each of the organic standards (DL-Malic, tartaric acid, oxalic acid and citric acid) were injected into HPLC and their chromatographic peaks were obtained. After injection of the samples, chromatographic peaks were identified by comparing the retention times of the samples with those of the known standards. The quantities of organic acids were estimated from the peak areas from injecting the known amounts of the standards.

# 3. Results

As seen in Table 1, the total phenolic content varied from 63.90 mg/100g to 381.23 mg/100g of among the three fruits tested. The highest phenolic content was found in *Salacca zalacca*, where the phenolic content in its young, mature, and ripe stage was at 381.23 mg/100g, 274.56 mg/100g and 324.90 mg/100g respectively. Meanwhile, the phenolic content for *Mangifera indica* L. Chok Anan in young, mature and ripe stage were at 180.90 mg/100g, 106.23 mg/100g, 149.23 mg/100g respectively. *Baccaurea motleyana* Hook. F. was having the lowest total phenolic content among the three fruits selected for this study. The total phenolic content in *Baccaurea motleyana* Hook. F. young, mature and ripe stages were only at 97.23 mg/100g, 63.90 mg/100g and 79.57 mg/100g respectively.

The total phenolic content was the highest in young stage for each fruit, and the phenolic content significantly decreases ($P<0.05$) as the fruit transformed into maturity. However, the phenolic content increases significantly ($P<0.05$) when it progressed into ripeness. Despite the increase that occurred from mature to ripe stage, the phenolic content in ripe stage is still significantly lower ($P<0.05$) when compared to young stage of fruit.

Table 1. Total Phenolic Content in selected fruits at different maturity stages

| Fruit Maturity Stages | *Salacca zalacca* (SALAK) | *Mangifera indica L. Chok Anan* (MANGO) | *Baccaurea motleyana Hook. F.* (RAMBAI) |
|---|---|---|---|
| Young | 381.23 ± 2.52 $^{a}$ | 180.90 ± 2.65 $^{d}$ | 97.23 ± 3.79 $^{g}$ |
| Mature | 274.56 ± 3.21 $^{b}$ | 106.23 ± 2.52 $^{e}$ | 63.90 ± 1.73 $^{h}$ |

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| | | | |
|---|---|---|---|
| Ripe | 324.90 ± 3.46 $^{c}$ | 149.23 ± 0.58 $^{f}$ | 79.57 ± 3.06 $^{i}$ |

Note: Results are represented by mean ± standard deviation (n=10). Means with different superscript letters are significantly different at P<0.05 (Tukey test). Data expressed as mg of gallic acid equivalent (GAE) per 100 g of fruit.

According to Table 2, the highest antioxidant activity occurred in *Salacca zalacca*. The percentage (%) of inhibition for *Salacca zalacca* in young, mature and ripe stage was at 84.45 %, 81.40 % and 83.85 % respectively. *Salacca zalacca* in young stage had a significantly higher (P<0.05) antioxidant activity compared to mature and ripe stages.

The percentage (%) of inhibition for *Mangifera indica* L. Chok Anan at young, mature and ripe stage of was at 66.23 %, 51.93 % and 54.83 % respectively. Although the range for percentage (%) of inhibition among young, mature, ripe stage is small, there were significant differences (P<0.05) in the three maturity stages of this fruit. Similar to *Salacca zalacca*, the antioxidant activity of fruit in young stage is significantly higher, whereas the antioxidant activity decreased significantly when the fruits matured and ripen.

The lowest antioxidant activity was obtained for *Baccaurea motleyana* Hook. F. The percentage (%) of inhibition for its young, mature and ripe stage was only at 13.10 %, 3.10 % and 6.47 % respectively. The antioxidant activity in *Baccaurea motleyana* Hook F. mature stage was significantly lower (P<0.05) than young and ripe stage. The antioxidant activity increased significantly (P<0.05) from ripe to young stage. The increasing pattern in antioxidant activity was found similar with *Salacca zalacca* and *Mangifera indica* L. Chok Anan with different maturity stages.

Table 2. Antioxidant (DPPH scavenging) activities of selected fruits at different maturity stages.

| Fruit Maturity Stages | *Salacca zalacca* (SALAK) | *Mangifera indica L. Chok Anan* (MANGO) | *Baccaurea motleyana Hook. F.* (RAMBAI) |
|---|---|---|---|
| Young | 84.45 ± 0.19 $^{a}$ | 66.23 ± 0.43 $^{d}$ | 13.10 ± 0.34 $^{g}$ |
| Mature | 81.40 ± 0.33 $^{b}$ | 51.93 ± 0.62 $^{e}$ | 3.10 ± 0.59 $^{h}$ |
| Ripe | 83.85 ± 0.16 $^{c}$ | 54.38 ± 0.34 $^{f}$ | 6.47 ± 0.66 $^{i}$ |

Note: Results are represented by mean ± standard deviation (n=10). Means with different superscript letters are significantly different at P<0.05 (Tukey test). Data expressed as percentage (%) of DPPH inhibition.

As seen in Table 3, *Salacca zalacca* showed the largest capacity to achieve 50 % of DPPH inhibition. The IC50 for *Salacca zalacca* at young stage was at 0.57 mg/ml, followed by ripe and mature stage, with an IC50 value of 1.63 mg/ml and 2.38 mg/ml respectively. *Mangifera indica* L. Chok Anan showed moderate capacity to achieve 50 % of DPPH inhibition, with IC50 value of 6.85 mg/ml, 9.24 mg/ml and 8.63 mg/ml as the fruit maturing and ripening. However, the IC50 for *Baccaurea Motleyana* Hook. F. cannot be determined due to low antioxidant activity thus 50 % of DPPH inhibition was not achieved.

Table 3. $IC_{50}$ values (50% DPPH inhibition) in selected fruits at different maturity stages

| Fruit Maturity Stages | *Salacca zalacca* (SALAK) | *Mangifera indica L. Chok Anan* (MANGO) | *Baccaurea motleyana Hook. F.* (RAMBAI) |
|---|---|---|---|
| Young | 0.57mg/ml | 6.85mg/ml | - |
| | | | |
| Mature | 2.38mg/ml | 9.24mg/ml | - |
| Ripe | 1.63mg/ml | 8.63mg/ml | - |

Note: Data were calculated according to figure 4.4 by regression equation.
“ - ” represent no $IC_{50}$ achieved for the fruit sample.

The correlation between antioxidant activity and total phenolic content of selected fruits in different maturity stages is shown in Fig 1. The result showed a positive linear correlation with $R^2 = 0.7761$. This result indicated that the phenolic compounds are an important factor which contributes to the antioxidant activities of fruits.

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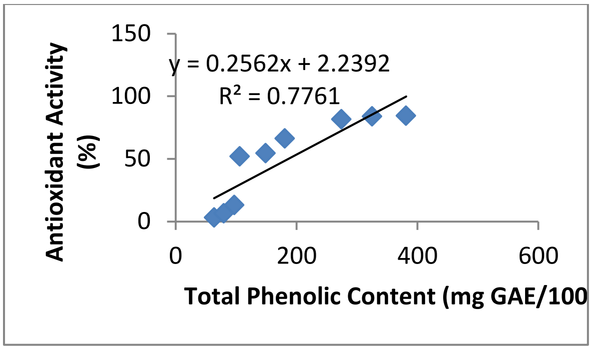


Fig 1. Correlation between antioxidant activities and total phenolic content of the three fruits analyzed.

As shown in Table 4, *M. indica* L. Chok Anan contained malic acid as the dominant organic acid at the young, mature and ripe stages followed by citric acid. For *B. motleyana* Hook. F., citric acid was the dominant organic acid at the young, mature and ripe stages, followed by tartaric acid, malic and oxalic acid. For the *S. zalacca*, malic acid was the dominant organic acid at the young, mature and ripe stages, followed by citric acid, tartaric and oxalic acid.

Table 4. Organic acid content (mg/ml) in the selected fruits at different maturity stages

| organic Acid | Stages | *Mangifera indica L. Chok Anan* (Mango) | *Baccaurea motleyana Hook. F.* (Rambai) | *Salacca zalacca* (Salak) |
|---|---|---|---|---|
| Citric Acid | Young | 97.247±0.516$^{a}$ | 30.551±1.197$^{b}$ | 16.419±5.419$^{c}$ |
| | Mature | 87.219±4.462$^{a}$ | 26.206±0.503$^{b}$ | 8.406±0.640$^{c}$ |
| | Ripe | 13.140±7.888$^{a}$ | 23.242±1.541$^{b}$ | 5.450±1.675$^{a}$ |
| Malic Acid | Young | 400.850±52.344$^{a}$ | 2.74±0.044$^{b}$ | 89.629±26.052$^{c}$ |
| | Mature | 312.440±4.803$^{a}$ | 3.425±0.047$^{b}$ | 62.490±29.101$^{c}$ |
| | Ripe | 219.650±1.974$^{a}$ | 3.144±0.089$^{b}$ | 12.781±7.154$^{b}$ |
| Tartaric acid | Young | 0.582±0.040$^{a}$ | 21.827±1.207$^{b}$ | 0.489±0.0628$^{a}$ |
| | Mature | 0.834±0.041$^{a}$ | 20.353±0.552$^{b}$ | 0.413±0.101$^{a}$ |
| | Ripe | 0.945±0.035$^{a}$ | 18.248±0.585$^{b}$ | 0.253±0.075$^{a}$ |
| Oxalic Acid | Young | 0.675±0.023$^{a}$ | 0.991±0.039$^{b}$ | 0.675±0.023$^{a}$ |
| | Mature | 0.975±0.054$^{a}$ | 0.975±0.054$^{a}$ | 0.916±0.967$^{c}$ |
| | Ripe | 0.689±0.005$^{a}$ | 0.926±0.105$^{b}$ | 0.689±0.005$^{a}$ |
| Total Acid | Young | 499.354±173.156$^{a}$ | 56.109±13.142$^{b}$ | 107.212±40.120$^{b}$ |
| | Mature | 401.468±133.106$^{a}$ | 50.959±11.259$^{b}$ | 74.204±29.733$^{b}$ |
| | Ripe | 234.424±97.265$^{a}$ | 45.56±10.002$^{a}$ | 19.173±6.128$^{a}$ |

Value (mean±SD) are average of three samples of each fruits, analyzed individually in triplicate ($p<0.05$). Different superscript letters in the same column represent significant differences among ripening stages per individual organic acid.

## 4. Discussion

The present study was conducted to determine the total phenolic content, antioxidant activity and organic acids of *Salacca zalacca*, *Mangifera indica* L. Chok Anan and *Baccaurea motleyana* Hook. F. at different maturity stages. Basically, total phenolic content and antioxidant activity in the three fruits varies irregularly when maturity progressed. Result suggest that the highest total phenolic content and antioxidant activity was both found in *Salacca zalacca*, whereas young stage of *Salacca zalacca* contains the highest amount of total phenolic content (381.23 ± 2.52 mg GAE/100g) and antioxidant activity (84.45 ± 0.19 %). This finding is in line with Ong & Law (2012), who stated that *Salacca zalacca* is a fruit that contain the highest amount of total phenolic compounds compared to several fruits such as mango, kiwi, fuji apple and mangosteen. There was no study found regarding the relationship between antioxidant activity of *Salacca zalacca*, *Mangifera indica* L. Chok Anan and *Baccaurea motleyana* Hook. F. being influenced by maturity of the fruits. However, the present study is in contrast with the recent study conducted by Zuhair *et al.*, (2013) who stated that the antioxidant activity in papaya fruit increases as ripening stage progressed and study by Nur Rahman *et al.* (2013) who stated

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that old and dried 70 % ethanol extract of *Solanum tarvum* has the highest amount of antioxidant than young and fresh fruit.

The inhibitory concentration (IC50) value for young *Salacca zalacca* is the lowest at 0.57 mg/ml, indicating that it contains high level of antioxidants. In addition, there was a positive correlation occurred ($R^2$ = 0.7761) between total phenolic content and antioxidant activity in this study. Tosun *et al.* (2009) stated that phenolic content in plants may directly contribute to their antioxidant activity. Several researchers (Borneo *et al.*, 2008; Ikram, 2009 ; Li Fu *et al.*, 2010) have reported the antioxidant activity in fruit is being influenced by total phenolic compound.

Generally, the composition of organic acids decreased as fruit maturity progressed so as the antioxidant activity of the fruits. *Mangifera indica* L. Chok Anan mango was found to contain the highest and most variety of organic acid. The total concentration of organic acid in young, mature and ripe stages were at 499.354 mg/ml, 401.468 mg/ml and 234.424 mg/ml respectively. Vanida, *et al.*, (2006) studied the organic acid in tropical fruit *Aglaia dookoo* Griff. at ripe stage and showed that citric acid and malic acid were present at the level of 0.22 % w/w and 0.15% w/w. Tahir *et al.*, (2012) reported citric acid as the major organic acid in fully ripened strawberry and mulberry genotype fruit. Nergiz *et al.*, (2010) reported that malic acid is the dominant acid in various types of olive fruit such as Memecik, Domat and Akhisar Uslu varieties in ripe stage. This result is contrast to the recent studied by Tahir *et al.*, (2012) reported that organic acids in foreign fruit such as strawberry, sweet cherry, and mulberry increased as maturity progressed. Usenik *et al.* (2008) evaluated sugar, total phenolic content, antioxidant activity and organic acid content of sweet cherry cultivars of different pomological characteristics and different time of ripening and found out that the sum of sugars (glucose, fructose, sucrose and sorbitol) ranged from 125 to 265 g/kg fresh weight (FW) and the sum of organic acids (malic, citric, shikimic and fumaric) ranged from 3.67 to 8.66 g/kg FW. Total phenolic content ranged from 44.3 to 87.9 mg GAE equivalents/100 g FW and antioxidant activity ranged from 8.0 to 17.2 mg ascorbic acid equivalent antioxidant capacity mg/100 g FW. They concluded that the correlation of antioxidant activity with total phenolic content and content of anthocyanins was cultivar dependent. Kelebek *et al.* (2009) identified three organic acids in orange juice and wines: citric, ascorbic and malic acids. He pointed out citric acid was the major organic acid in orange juice (12.66 g $L^{-1}$) and wine (6.03 g $L^{-1}$).

## 5. Conclusion

By conducting this research, we were able to determine that maturity stage of fruit will influence the total phenolic content and antioxidant activity in the three fruits. The highest TPC and antioxidant activity were found in the young stage of *S. zalacca* fruit extract with lowest inhibitory concentration (IC50) valued at 0.57 mgml-1. This research suggested that for these three fruits, a young fruit give a better source of total phenolic content and antioxidants. Moreover, we were able to obtain a positive linear correlation between the TPC and antioxidant activities of the fruit extracts. On the other hand, the tested fruits showed a decrease in the concentration of organic acids with ripening. Malic acid is the predominant organic acid in *M. indica* L. Chok Anan and *S. zalacca*, while citric acid is the predominant organic acid in *B. motleyana* Hook. F. This study however is limited to three tropical fruits only. More work should be done to study the relationship of fruit maturity with total phenolic compound, antioxidant and organic acid composition in other local fruits in Malaysia to understand their relationship further in order to advice the public on the health benefit of eating fruit at the right stage to get the best nutrition.

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