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# DETERMINATION AND COMPARISON OF THE TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND THE ANTIOXIDANT ACTIVITY OF GARCINIA MANGOSTANA L. (MANGOSTEEN) FRUIT IN SRI LANKA

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## *ABSTRACT*

Garcinia mangostana (Mangosteen) is a Southeast Asian tropical fruit, which is known for its edible pulp. Bioactive compounds of mangosteen have been applied evidently in traditional medicine for various motives. Objective of this study was to determine and compare the phenolic and flavonoid phytochemical contents and the antioxidant activities of the peel, rind, pulp, and seed extracts of the mangosteen fruit. The samples were dried using a dry-oven method at 40ºC, followed by phytochemical extraction with 70% methanol solution. The total phenolic content (TPC) was evaluated by Folin-Ciocalteu method, which ranged from 8.56 ± 1.17 µg/mL (rind) to 2.47 ± 0.29 µg/mL (seed), and the total flavonoid content (TFC) was evaluated by AlCl3 method, which ranged from 9.64 ± 0.65 µg/mL (rind) to 6.32 ± 0.34 µg/mL (seed). The antioxidant potentials evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) methods. Observed DPPH activity ranged from 95.22 ± 1.11 % (rind) to 61.82 ± 0.86 % (pulp) and observed ABTS activity ranged from 98.31 ± 0.70 % (rind) to 77.42 ± 3.02% (pulp). Antioxidant activities exhibited a positive correlation with both TPC and TFC while TFC were more responsible for the antioxidant activity than the TPC of the fruit. Furthermore, it demonstrated that the peel, rind and seed which is considered as waste, had more phytochemicals (phenolic acids and flavonoids) with higher antioxidant activities than the pulp which solely consumed.

Keywords: Garcinia mangostana, mangosteen, phenolic content, flavonoid content, antioxidant activity

## *INTRODUCTION*

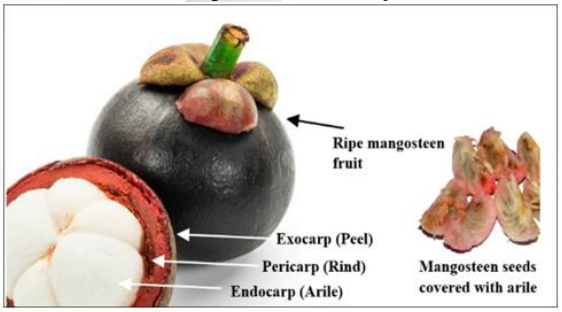
A free radical is a molecule potent of independent existence with an unpaired electron in an atomic orbital. It could provide or accept an electron from neighbouring molecules behaving as an oxidant or reductant (Halliwell and Gutteridge, 2015). Free radicals generated during metabolism gets balanced with the endogenous antioxidant defence system. However, diminished endogenous defence significantly contributes to deteriorating the defence mechanisms by damaging initial biomolecules including DNA, lipids, and proteins. Meanwhile, accelerating ischemic attacks, inflammatory diseases, hemochromatosis, emphysema, acquired immunodeficiency syndrome, and many other diseases (Ichiishi et al., 2016). Reactive oxygen species (ROS) predominantly cause cell damage and act as a physiological secondary messenger in signal transduction pathways (Bartosz and Kolakowska, 2011; Halliwell and Gutteidge, 2015). Thus, it demands the importance of antioxidants in scavenging radicals, which are involved in the pathogenesis of oxidative stress-related diseases.

According to the hypothesis of 'increased intake of antioxidants enables prevention from oxidative damage/stress and lower the risk of chronic diseases', researches are conducted to discover antioxidants (Stanner et al., 2004; Zarcovic, 2018). Currently, there are two main categories of antioxidants namely natural and synthetic. Since the synthetic antioxidants have reported multiple long-term adverse effects including toxicity and carcinogenicity, studies over the past decade have focused on natural antioxidants over synthetic (Troncoso et al., 2005). Therefore, plants have gained scientific interest by containing natural antioxidant constituents such as phenolic acids. Antioxidants in dietary supplements are demanding over synthetic antioxidants not only due to fewer side effects but also, they equally act against reactive species (ROS and NOS), preventing oxidative stress.

Garcinia mangostana L. (Mangosteen, Clusiaceae) is one such Southeast Asian tropical fruit, known to have a delightful edible pulp. The edible pulp is only 30% of the total fresh weight of the fruit, while the remaining 70% is considered as waste (Osman et al., 2006). Bioactive compounds of mangosteen have been evidently used in traditional medicine in Sri Lanka with various therapeutic motives. The pericarp (referred as rind) extract is well-known traditional folk-medicine reported with antioxidant, antimicrobial, antimalarial, and anti-inflammatory activities due to its presented functional compounds (Sakagami et al., 2005; Cui et al., 2010; Aisha et al., 2012).

Addressing the emerging public demand for natural antioxidants-rich food products, the present study aimed to determine and compare the phytochemical (phenolic acids and flavonoids) content and its antioxidant activities. Furthermore, there are inadequate researches done on comparing all the parts of mangosteen in a simgle study. Henceforth, this study was designed to compare the phenolic and flavonoid content of each part of the fruit (peel, rind, pulp, and seed) separately and their respective antioxidant activity levels.

Figure 1 - Parts of the mangosteen fruit. The exocarp (peel), pericarp (rind, endocarp (arile/pulp), and seeds were obtained as the samples for the study.




In Sri Lanka, mangosteen cultivation dates back to 1800s (Gary and Ender, 1996). Estimations showed that over 33,000 trees producing over 11 million fruits in seven main districts including Kalutara, Ratnapura, and Kandy which accounted for nearly 75% of total production (Agribusiness and Marketing Assistance Service, 2000; Agarian Research and Training Institute, 2003). Due to the abundance of Mangosteen and the absence of pest attacks and diseases in Sri Lanka, ripe mangosteen fruits from Kalutara district were obtained as the samples for this project.

Extraction of phytochemicals from an organic sample mainly depends on the solvent and the technique; in which the polar solvents are used frequently to extract polyphenols. Henceforth, conventional aqueous solvent (ethanol, methanol, ethyl acetate and acetone) based extraction was followed. Although ethanol is widely used as an extraction solvent and safe for human consumption, methanol has shown more efficient in extracting

phenols. Hence, the total phytochemical extraction of the study followed a methanolic extraction.

The general objective of the study is to quantify and compare the phenolic and flavonoid contents of the desired parts of G. mangostana fruit and to determine antioxidant activity of the extracted samples. The total phenolic content (TPC) and total flavonoid content (TFC) were evaluated by the Folin-Ciocalteu and the aluminium chloride (AlCl3) colorimetric based techniques while antioxidant activity was estimated by two assays, 1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2-Azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) free radical cation scavenging activity by methanolic extraction respectively. The antioxidant activities of the two methods were compared and the correlation between the phytochemical content with respect to its antioxidant activities were evaluated.

## *METHODOLOGY*

Sample collection and preparation

Fresh and mature mangosteen fruits were obtained from Kalutara district, Sri Lanka. Fruits were cleaned out of dirt, washed with water and drained. They were sliced crosswise and the peel, rind, arile, and seeds were separated and cut into pieces respectively as shown in the Figure 1. Parts were dried at 40ºC in the dry oven until they were fully dried. Dried parts were ground into find power and stored at room temperature (RT) in tightly closed containers.

**Extraction of phytochemicals**

Extraction of phytochemicals was adapted from Lim and colleagues (2013), with minor modifications. Four grams of each powdered sample were weighed separately and mixed with 400mL of 70% methanol. Aliquots were placed in a water bath at 40ºC for three hours. Samples were then centrifuged at 4000rpm for 10 minutes and the supernatant was collected to prepare extraction solution for each sample separately (Lim et al., 2013). The supernatant was evaporated at 40ºC until solid-gel was formed. Evaporate was then scraped, weighed, and stored at -4ºC for further use. Working solutions were prepared by mixing 10mg of the stock with 100mL of 70% methanol to obtain a concentration of 100µg/mL.

**Total phenolic content (TPC) and total flavonoid content (TFC)**

TPC was determined by using the Folin-Ciocaltau (FC) method (Cheok et al., 2011; Hiranrangsee et al., 2016). One millilitre of aliquot (triplicate) was mixed thoroughly with 5mL of diluted FC reagent (1:10). After letting to stand for three minutes, 4mL of 7.5% Sodium carbonate (Na2CO3) was added, mixed thoroughly and kept for 30 minutes in the dark prior to measuring the absorbance at 765nm against a blank of 70% methanol.

TFC was determined by aluminium chloride method according to Chang and colleagues (2014) with slight modifications. Two millilitres of the sample was mixed with 0.1mL of 10% (w/v) AlCl¬3, 0.1ml of 0.1mM Potassium acetate and was let to stand at RT for 30 minutes. The absorbance was measured at 415nm against a blank of 70% methanol (Chang et al., 2001).

Absorbance values were referred against a standard curve of Gallic acid and Rutin in TPC and TFC assays, respectively with known concentrations prepared with 70% methanol. Values were obtained in milligrams of Gallic acid equivalents (GAE) per millilitre and Rutin equivalents per millilitre, respectively.

**DPPH and ABTS scavenging (antioxidant) activities**

Two different antioxidant assays (DPPH and ABTS) were performed

according to the methods described by Witternawer and colleagues (2016) and Zheleva-Dimitrova and colleagues (2016) respectively.

Fresh 0.3mM DPPH solution was prepared in a methanolic solution. Afterwards, 2.5mL of each extract was mixed with 2.5mL of DPPH solution and was left at RT in the dark for 30 minutes before measuring the absorbance at 517nm. Percentage inhibition was calculated as shown in equation 1.

"Percentage inhibition by DPPH =" ( "A" _"control " "- " "A" _"sample" )/"A" _"control" " ×100% (1)"

Where Acontrol is the absorbance of DPPH without the extract and Asample is the absorbance of the sample with DPPH (Wittenawer et al., 2016).

A fresh ABTS solution was prepared by mixing 1mL of 7mM ABTS with 1mL of 2.4mM potassium persulphate solution (1:1). The mixture was kept in the dark for 12-16 hours at RT. The solution was diluted with methanol to obtain an absorbance of 0.706±0.01 at 734 using the spectrophotometer.

After the dilution, 1mL of each extract was mixed with 1mL of diluted ABTS solution and let to stand for seven minutes prior to measuring the absorbance at 734nm. Percentage of activity was determined as shown in equation 2.

$$\text{Percentage inhibition by ABTS} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (2)$$

Where A Control is the absorbance of ABTS radicals in methanol and A Sample is the absorbance of ABTS radicals of the extract mixed with methanol.

**Statistical analysis**

All experiments were carried out in triplicates and results were expressed as mean value with standard deviation (±SD) of the triplicates. One-way analysis of variance (ANOVA) was carried out to determine the significance of populations and difference between the groups were analysed by paired t-test by using the SPSS statistical software package (SPSS, version 22.0). The significance was determined at $p<0.05$.

## *RESULTS*

Total Phenolic Content

Table 1 – Mean concentrations of the total phenolic content of the samples

| Sample | Peel | Rind | Pulp | Seed |
|---|---|---|---|---|
| TPC (µg/mL) | 6.40 ± 0.63 | 8.56 ± 1.17 | 2.64 ± 0.10 | 2.47 ± 0.29 |
| TFC (µg/mL) | 7.08 ± 0.74 | 9.64 ± 0.65 | 6.56 ± 0.12 | 6.32 ± 0.34 |

According to Table 1, the highest concentration of TPC was observed in the rind followed by peel, pulp and seeds which was significant ($p=0.000$) at $p<0.05$. Phenolic contents within the samples were analyzed by the paired t-test. The TPC of the rind was 2.165 times higher than the peel, whereas the TPC of the peel was 10.869 times higher than the pulp. The TPC of the peel was 7.956 times higher than the seed, whereas the TPC of the rind was 8.073 times higher than the pulp. The TPC of the rind was 11.914 times higher than the seed and the TPC of the pulp was 0.787 times higher than the seed. The highest difference among the samples was observed in the rind-seed pair, which was presented respectively, as the highest and the lowest TPC values of the sample population. None of the pairs showed a significant difference at $0<0.05$.

According to Table 1, the highest concentration of TFC was observed in the rind followed by the peel, pulp, and seeds

which was significant (p=0.000) at p<0.05. Flavonoid contents within the samples were analyzed by the paired t-test. The TFC of the rind was 2.828 times higher than the peel, whereas the TFC of the peel was 10.730 times higher than the pulp. The TC of the peel was 7.892 times higher than the seed, whereas TFC of the rind was 14.517 times higher than the pulp. The TFC of the rind was 36.651 times higher than the seed and the TFC of the pulp was 0.783 times higher than the seed. The highest difference among samples was observed in the rind-seed pair which was presented respectively as the highest and the lowest TFC values in the sample population. The difference between the rind and seed, and the rind and pulp extracts were significant (p=0.01, p=0.05 respectively) at p<0.05.

**Compared TPC and TFC**

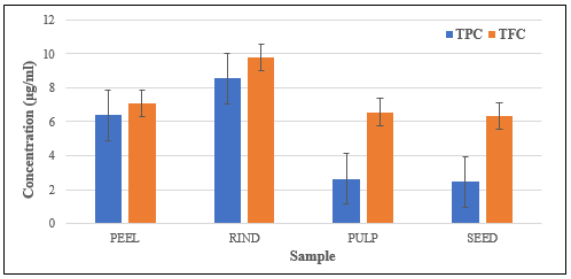



Figure 2 – *Compared TPC and TFC values of the samples.*

The phenolic and flavonoid concentrations of the samples compared in Figure 2 showed higher flavonoid content than the phenolic content in each sample. The difference between the TPC, TFC of samples were significant at p<0.05. As shown in Figure 3, a positive correlation was observed between TPC and TFC which was not significant at p<0.05.

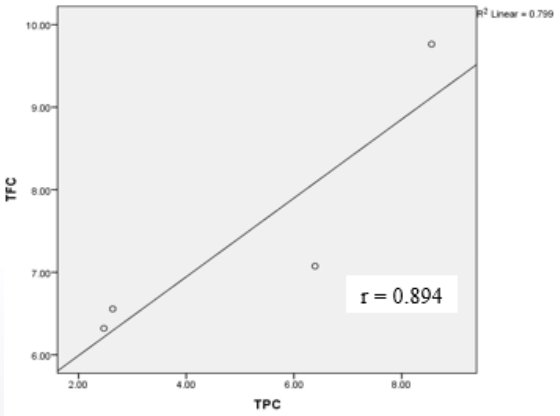



Figure 3 – *Correlation between TPC and TFC*

DPPH and ABTS Scavenging Activity

Table 2 – Mean percentage of DPPH and ABTS activity of the samples

| Sample | Peel | Rind | Pulp | Seed |
|---|---|---|---|---|
| DPPH (%) | 92.35 ± 2.13 | 95.22 ± 1.11 | 61.82 ± 0.86 | 94.06 ± 0.42 |
| ABTS (%) | 98.00 ± 1.06 | 98.31 ± 0.70 | 77.42 ± 3.02 | 98.13 ± 0.50 |

According to Table 2, the highest DPPH scavenging activity was observed in the rind followed by seed, peel and the pulp which was significant (p=0.000) at p<0.05. The DPPH activity within the samples were analyzed by the paired t-test. The DPPH activity of the rind was 2.87 times higher than the peel, whereas the DPPH of the peel was 30.54 higher than the pulp. The activity of the seed was 1.7 Times higher than the peel, whereas the activity of the rind was 33.40 times higher than the pulp. The activity of the rind was 1.17 times higher than the seed, whereas the activity of the pulp was 32.24 higher than the seed. The highest difference among the samples was observed in the rind-pulp extract pair, which presented respectively as the highest and the lowest DPPH activity values in the sample population. The difference between peel-

pulp, rind-pulp, and seed-pulp pairs were significant at $p<0.05$.

According to Table 2, the highest ABTS activity was observed in the rind followed by the peel, seed, and the pulp in a descending manner ($p=0.000$) at $p<0.05$. The ABTS activity within the samples were analysed by the paired t-test. ABTS activity of the rind was 0.32 times higher than the peel, whereas the ABTS activity of the peel was 20.58 times higher than the pulp. The ABTS activity of the seed was 0.46 times higher than the peel, whereas the ABTS activity of the rind was 20.90 times higher than the pulp. The ABTS activity of the seed was 0.15 times higher than the rind, whereas the ABTS activity of the seed was 21.04 times higher than the pulp. The highest difference among the samples was observed in the rind-pulp pair, which was presented respectively as the highest and the lowest ABTS activities in the sample population. The difference between the peel-pulp, and pulp-seed pairs were significant at $p<0.05$.

**Comparison between DPPH and ABTS percentage activities**

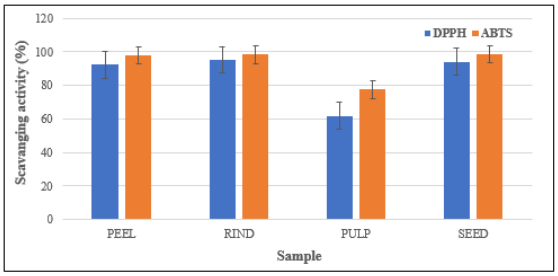



Figure 4 – *Compared DPPH and ABTS activities of the samples*

The DPPH and ABTS percentage activities compared in Figure 4, showed a higher ABTS activity than a DPPH activity in each sample extract (ABTS, DPPH; Peel- 98.00%, 92.35%; Rind- 98.31%, 95.22%; Pulp- 77.42%, 61.82%; Seed- 98.13%, 94.06%).

**Correlation between the phytochemical content and antioxidant activities**

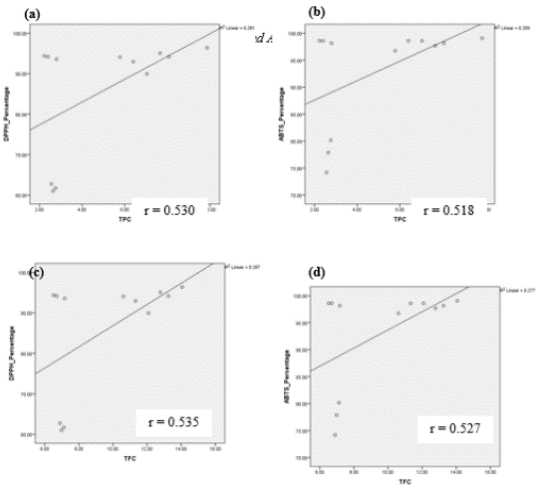



Figure 5 – Pearson's correlation of DPPH, ABTS with TPC and TFC. (a) Correlation between TPC and DPPH; (b) Correlation between TPC and ABTS; (c) Correlation between TFC and DPPH; (d) Correlation between TFC and ABTS.

As shown in Figure 5, a positive correlation was observed between DPPH and TPC which was not significant ($p=0.076$) at $p<0.05$. A positive correlation was observed between ABTS and TPC which was not significant ($p=0.084$) at $p<0.05$. A positive correlation of DPPH and TFC was observed which was not significant ($p=0.073$) at $p<0.05$. A positive correlation of ABTS and TFC was observed which was not significant ($p=0.079$) at $p<0.05$. Furthermore, TFC showed higher correlation in both DPPH and ABTS assays compared to TPC.

## *DISCUSSION*

Free radicals are normally generated during metabolism, which is balanced with the endogenous antioxidant defence system (Haliwell and Gutteridge, 2011). However, diminished endogenous defence leads to oxidative stress damaging the initial biomolecules. Thus, it is clear that the oxidising of these biomolecules should be prevented by antioxidants. The antioxidants are crucial in scavenging oxidative radicals involved in the pathogenesis of oxidative stress related diseases (Rufino et al., 2010). Although scientists have developed synthetic antioxidants, they also have reported with several long-term effects including toxicity and carcinogenicity. As an alternative source, plants have gained scientific interest as they contain natural antioxidant constituents such as phenolic acids. According to literature, antioxidants in dietary substance are able to act against the effect of reactive species (ROS and RNS) preventing oxidative stress (Monsen, 2000). With the arising awareness of functional foods for health, especially those containing antioxidants have resulted in extensive research on phytochemicals.

Mangosteen is one such fruit that is evident to have antioxidant activity. It is known to have a delightful edible pulp, however the rest of the fruit (peel, rind, and the seed) which comprises of 70% of the fruit considered as waste by consumers. In this study, the phytochemical composition and the antioxidant activity of each part of the mangosteen fruit were separately extracted and evaluated.

Several studies have emphasized the importance of the sample preparation and the extraction method in the determination of phenolic compounds and antioxidants as the responses vary for different methods. This initially depends on the nature and the chemical properties of the sample including the concentration and the polarity (Khodammi et al., 2013). The phenolic content of a fruit influenced by its degree of maturity at harvest, pre-harvest environmental conditions, post-harvest storage conditions and processing (Shahidi and Naczk, 2004). The samples were assured to be influenced by same environmental conditions and same maturation levels. In sample preparation, freeze-drying, air-drying or oven-drying is normally used. Sejali and Anur (2011) demonstrated that higher quantities of phenol were extracted by shade air-dried neam leaves over oven-dried samples. However, this study followed the oven-dry method to avoid possible environmental contaminations and microbial interactions. Optimal drying temperature should be evaluated since it can affect the quality and properties of the extract as the components tend to degrade in high temperature (Mulia, et al., 2018). Samples were dried at 40ºC for seven days since the pulp contained a high amount of moisture although previous studies have shown effective drying temperature for mangosteen hull is 65ºC for 30 minutes (Satong-aun et al., 2011). A higher phenol extraction can also be achieved by grinding or milling the sample as it improves the enzymatic activity of the samples giving rise to its metabolites. Thus, the samples were ground into a find powder.

Several parameters influence the phytochemical extraction from the samples such as temperature, extraction time, solvent-sample ratio, solvent type, and several repeat extraction of the sample (Shaidi and Naczk, 20004). Example, undesirable enzymatic oxidations and degradations are possible with increased temperature and extended time. Hence, the RT was used for the phytochemical extraction, as it was responsible for less solvent loss and favourable for heat-sensitive compounds preventing degradation, which is advantageous.

Numerous extraction methods have suggested to obtain phytochemicals mixing with several organic solvents including acetone, ethanol, ethyl acetate, hexane and methanol. Ethanol and methanol are commonly used extraction solvents in phytochemical extraction in magosteen. Recently, a research was conducted to investigate different solvents in extracting phenolic compounds from mangosteen hull (peel and rind) and it concluded methanol as the most suitable solvent for the TPC assay (Cheok et al., 2011). It further showed how well the solvent can insulate the opposite charge from one another depending on its dielectric constant while the TOPC yield is directly proportional to its dielectric constant. Comparatively, methanol has a higher dielectric constant (32.7 at 25ºC) compared to other organic solvents (24.5 of Ethanol; 21 of Acetone; 6 of Ethyl acetate at 2525ºC) resulting in higher TPC values (Bruice, 2007). The yield also depends on the solvent polarity and the phenolic compound and chemical structure of the solvent (Cheok et al., 2011).

Methanol also reported having the ability to inhibit the polyphenol oxidase preventing polyphenol oxidation (Yao et al., 2004). Considering the optimal methanol concentration, Cheok and colleagues (2012) reported 70% methanol as the optimal concentration for methanol in TOC extraction. Thus, 70% methanol was used for the phytochemical extraction in the current study. The sample extracts proceeded to both phenol and flavonoid quantification. Mangosteen produces a diverse range of phenolic metabolites such as flavonoids, anthocyanins and tannins. The quantification methods of phenols are mostly based on spectrometric methods where the sample is reacted with a colorimetric reagent and observed under the visible range of the spectrum. They are rapid, easy and applicable for routine quantifications. The Folin-Ciocalteu (F-C) method is one such simple and reproducible method which is used routinely to quantify, quality control and determines antioxidant activities especially in food and dietary products (Prior, Wu & Schaich, 2005). Theoretically, the electrons from phenolic compounds transfer to phosphomolybdic/ phosphotungstic acid complexes in an alkaline medium, which was determined at 765nm by the formation of blue coloured complexes (Singleton et al., 1999).

The rind of the fruit showed the highest phenolic content followed by the peel, pulp and seed. The rind was 3-folds higher than the pulp and seed, respectively while the peel was 2-folds higher than the pulp and seed, respectively. The observed pattern was further in agreement with previous studies, which reported that the highest TPC in the mangosteen hull compared to its pulp (Okonogo et al., 2007; Zadernowski et al., 2009; Cheok et al., 2011; Naczk et al., 2011). Mangosteen fruits are also rich in xanthones, anthocyanins and condensed tannins (CT) also known as proanthocyanidins (Zadernowski et al., 2009). Currently over fifty xanthones were isolated and identified in the hull of the fruit (Jung et al., 2006). Furthermore, Naczk and colleagues (2011) determined the distribution of CT by vanillin and proanthocyanidin assays, which reported significantly higher CT in the rind and peel compared to the pulp.

Flavonoids are highly bioactive phenolic compounds present in mangosteen, which is widely used, in traditional medicine due to its antibacterial, antihypersensitive, antioxidant, and various other pharmacological effects (Hasan et al., 2016). The most common method of quantifying flavonoids is the AlCl3 based colorimetric assay which the flavonoids react with NaNO2-Al(NO3)3-NaOH (Jia et al., 2015). Methanolic extract with AlCl3 allows quantification of flavonoids

at 410-423nm range (Huang et al., 2009). The highest flavonoid content was observed in the rind followed by the peel, pulp and seed which demonstrated the same pattern observed in TPC. The rind and pulp showed a significant difference in TFC such that comparatively higher TFC was presented in the rind compared to the pulp. This pattern was observed in previous studies (Naczk et al., 2011).

In the comparison of TPC with TFC, higher TFC values were observed compares to its respective TPC values, which were contradictory with the literature (Zarena and Sankar, 2011). Normally, the TPC should be higher than the TFC since flavonoids are a major compound, which is already present in the TPC. This may occur due to the methods used to quantify two assays which are F-C method and AlCl3 based method and their reference standards which are gallic acid and rutin respectively. Furthermore, it could be due to the extraction solvent which extracted flavonoids efficiently. Therefore, flavonoids appear as the principle constituent of the crude extract of the samples since the phytochemical extraction method by methanol is not 100% pure and efficient. Moreover, the F-C reagent tend to react non-specifically with aromatic amines, unanticipated phenols, high ascorbic acid or sugar levels (Singleton & Rossi, 1965; Everette et al., 2010). Variations of the phenolic acids due to environmental stress tends to cause low phenolic contents (Pasqualini et al., 2003). Also, the addition of the alkali (Na2CO3) and the competing antioxidant with F-C reagent are possible inhibitions by the oxidised substrates in the extracts. Thus, F-C reagent was added before the alkali to avoid air-oxidation. Considering the AlCl3 based method, TFC quantification is also not a highly specific method as it interferes with non-flavonoid components such as azo-dihydric phenols (Zhang et al., 2010; Qiu et al., 2013). Thus, higher TFC values than TPC could be resulted in non-specific interactions of flavonoids with NaNO2-Al (NO3)3-NaOH (Huang et al., 2009).

With the observed TFC and TPC values, antioxidant activities were evaluated in each extract. Thus, two independent methods DPPH and ABTS were used to compare the free radical scavenging activity/ reducing power of the extracts. DPPH• and ABTS•+ are non-physiological molecules which are used to determine the radical-scavenging activity by single electron transfer (SET) reaction (Olszowy and Dawidowicz, 2018).

DPPH assay is the primary method to determine the antioxidant activity of potential plant phenolic extract (Zarena and Sankar, 2009). DPPH is scavenged forming reduced form of DPPH (DPPH-H) by accepting a hydrogen atom released by an antioxidant (Wang et al., 2008). The highest adsorption of a stable radical in methanolic solution is at 515nm (Brand-Williams et al., 1995). However, studies reported it to be at 517nm (Naczk et al., 2011). Thus, DPPH activity was evaluated at 517nm. In this study, the direct DPPH activity of a fixed concentration was determined. It can be further evaluated with a concentration gradient of respective sample to obtain the antioxidant potential of the sample expressed as the half-maximal inhibitory concentration, IC50 (amount of antioxidants required to decrease the initial DPPH concentration by 50%) since the reduction of absorbance is depend on the quantity of the antioxidants, radicals and their kinetic behaviour.

ABTS is produced by reacting the ABTS salt with potassium permanganate or potassium persulphate. ABTS assay determines the relative ability of scavenging based on the generation of a blue/green ABTS• + that can be reduced by antioxidants (Floegel et al., 2011; Zhong and Shahidi, 2015). The characteristic wavelength for ABTS is at 734 nm. In the comparison of two

methods, better estimations were observed in the ABTS assay compared to DPPH. ABTS method can be used at different pH levels, unlike DPPH which is pH sensitive (Lin et al., 2013). Therefore, ABTS is preferred when studying the effect of pH with antioxidant activity (Widowati et al., 2014). Furthermore, the ABTS method was rapid compared to the DPPH method.

This study showed the same pattern of DPPH and ABTS activities of the samples. The highest scavenging activity was shown in the rind followed by the seed, peel and the pulp showed the lowest activity. The scavenging activity of the rind was 2-folds higher than the pulp in both assays. The scavenging activity of the rind was 2-folds higher than the pulp in both assays. This pattern was further agreed with previous studies, which reported 2-3 folds higher activity in the rind than the peel and the pulp (Naczk et al., 2011). Also, it was reported a 1.5 folds higher activity in the rind than the peel and pulp (Lim et al., 2013). Although seed extracts showed the least TPC and TFC values, a higher antioxidant activity was observed compared to the pulp. It also agreed with the available literature (Lim et al., 2013). TPC and TFC values were positively correlated with DPPH and ABTS activities which TFC showed a higher correlation to DPPH and ABTS compared to TPC. This further reasoned the observed higher TFC values than TPC values. Moreover, it showed that flavonoid content of each sample has contributed to its respective antioxidant activities in both DPPH and ABTS assays compared to its phenol content.

Lack of available literature on comparing all the parts of mangosteen was a limitation on comparing the current results because the researches were mainly focused on the edible pulp and the rind of mangosteen. There were limited number of previous research done on mangosteen peel and seeds. Therefore, it was difficult to make a direct comparison of the results of this study with the available research studies. This obstacle was mainly due to different methods of extraction, quantifications, standard curve reference solutions and antioxidant determination assays (Zadernowski et al, 2009; Ragasa et al., 2016; Saputri et al., 2018). Henceforth, development of an optimised and a standard protocol for mangosteen phytochemical extraction and evaluating antioxidants are crucial.

## *CONSLUSION*

It can be concluded that the highest phytochemical content (phenolic acids and flavonoids) observed in the rind which also showed the highest antioxidant activity in both DPPH and ABTS scavenging assays followed by the rind, pulp, and seed. This further highlighted that the peel, rind, and seed, which is considered as the waste has a significant content of phytochemicals with high antioxidant activities which might enhance the future utilization of the fruit waste in medical, pharmacological, and food industry dietary supplements.

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