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Extracts of Peel and Different Parts of MD2 Pineapple as Potent Nutraceuticals

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Introduction

Nutraceutical is referring to any nutrient or phytochemical that has therapeutic effect. An antioxidant is an example of nutraceutical which protects against potentially toxic and harmful species by increasing oxidative stability of the cellular system.[1] It neutralises free radicals that contain at least one unpaired electron in its outer orbit and capable of independent existence that mainly derived from oxygen and nitrogen. These free radicals are known as reactive oxygen and reactive nitrogen species. Free radical plays a major role in development of chronic diseases such as cancer, cardiovascular diseases, diabetes and neurodegenerative disorders.[2] In the food sector, lipid oxidation initiated by free radicals contributes to food deterioration, particularly food products which are high in lipid or lipid-containing foods, resulting in the development of undesired flavour and rancidity.[3] Therefore, consuming antioxidant-rich food such as fruit and vegetable is important for maintaining good health.

Pineapple (*Ananas comosus* L.) is a fruit which belongs to the Bromeliaceae family and grows in most tropical and subtropical countries. It contains a high amount of vitamin C, calcium, potassium and fiber.[4] There are many types of pineapple available in Malaysia. MD2 pineapple is a hybrid type that having high quality.[5] Despite the high-quality features of MD2 pineapple, there is a lack of study on different parts of pineapple, and these edible and non-edible parts have high antioxidant properties. However, most studies determined the antioxidants content in pineapple flesh. There is also no study examine and compare different parts of MD2 pineapple. Hence, it is important to study the bioactive compounds in pineapple flesh and its by-products for production of nutraceuticals as well as help in cost and environmental saving.

Methods

Sample preparation and extraction

The freshly purchased pineapple was prepared into three different parts which were flesh (with core), peel and core. The flesh, peel and core were freeze dried separately and blended into powder. A 3 g of each lyophilised sample was mixed with 80% ethanol at a ratio of 1:10 (w/v) and homogenised. The mixture was then centrifuged at 3000 rpm for 10 min and filtered to obtain a clear extract and the residue was re-extracted. The ethanol was removed before further analyses and extraction yield was calculated for each replicate of the sample. Moisture content of the fresh sample was determined using the air oven drying method as stated by AOAC Official Method 990.19.[6]

Determination of total phenolic and total flavonoid content

Total phenolic content (TPC) of each sample was determined using Folin-Ciocalteu reagent method with slight modification.[7] Briefly, 100 µL extract was added with a 1.5 mL Folin Ciocalteu reagent (diluted 10 times) and added with 1.2 mL of 7.5% w/v sodium carbonate. The mixture was allowed to stand for 30 min before absorbance was taken at 765 nm. The result was expressed as mg gallic acid equivalent (GAE) per g dry weight (DW).

Total flavonoid content (TFC) of the sample was determined using aluminium chloride method with slight modification.[8] Briefly, 100 µL extract was mixed with 2.25 mL of ethanol solution in a test tube followed by addition of 0.15 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added and finally added with 1.0 mL of 1 M NaOH after 6 min of incubation. Absorbance was measured at 510 nm and the result was expressed as mg quercetin equivalent (QE)/g DW.

Antioxidant activity assays

Antioxidant activities of pineapple extracts were performed according to DPPH method described by Krings and Berger.[9] Briefly,10 g of the sample was extracted and diluted. A 2 mL of 0.15 mM DPPH solution in ethanol was added to each extract. The mixture was left to stand for 30 min at room temperature before the absorbance was read at 517 nm. An ascorbic acid solution of different concentrations was used as an analytical standard.

Ferric reducing antioxidant power (FRAP) assay was performed and the FRAP solution was prepared according to procedures described in the literature with slight modification.[10] Pineapple extract (50 µL) was added to 950 µL of FRAP solution and incubated at room temperature for 45 min. The absorbance was read at 593 nm. FRAP value for each extract was calculated and expressed in mmol Fe (II)/kg DW.

Statistical analysis

All data were presented as mean ± standard deviation of three replicates using IBM SPSS Statistics version 24. One-way analysis of variance (ANOVA) was applied to compare the mean differences among the different parts of pineapple. Pearson correlation test was used to determine the correlations between the total phenolics and antioxidant activities at $p<0.05$.

Results and Discussion

Moisture content

The result showed that MD2 pineapple flesh had the highest moisture content (87.19 ± 0.35), followed by mixture (86.07 ± 2.61), core (84.56 ± 2.00%) and peel (78.42 ± 1.73%). There were significant differences between these parts of pineapple at $p<0.05$, except for flesh and mixture. In fact, moisture content of pineapple differs depending on the ripening period.[11]

Total phenolic content and total flavonoid content

As shown in Figure 1A, TPC of MD2 pineapple samples is in descending order: peel > flesh > mixture > core. Based on the statistical results, there were significant differences in TPC among the pineapple peel, flesh, mixture and core parts at $p<0.05$. The findings of current study are in line with the previous study done by Ding[12] who reported a relatively low TPC from 4.78 mg GAE/g to 5.00 mg GAE/g for the flesh of MD2 pineapple. The differences in phenolic content as compared with to previous studies may be due to the variation in plant cultivar as influenced by maturity stage, genetic factors and postharvest conditions.[13] Furthermore, the differences in planting locations also influence the growing temperature, environmental factors and climatic changes also affect the phenolic content.[14]

Figure 1B shows the TFC of MD2 pineapple samples in descending order: peel > flesh > mixture > core. There were significant differences in TFC between the different parts of the pineapple at $p<0.05$ while TFC between the flesh and mixture was not significantly different. A previous study supports our finding that flavonoids content in the outer layer of citrus is higher than the inner layer.[15] Also, the flavonoids content in the by-products was higher than the fruit pulp.[16] The finding could be due to the outer part of fruit is being exposed to sunlight and lead to the synthesis of phytochemicals such as flavonoids by the plant to protect its the fruit, whereas the absorption of light is converted into chlorophyll to generate energy.[17] The flavonoid profile has been shown to differ among species and cultivars of pineapple, region specialty, climate, soil characteristics and cultivation techniques.[18]

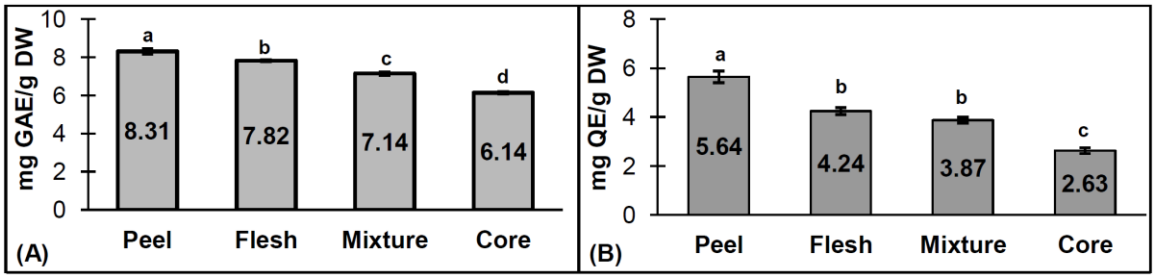

Figure 1 Total phenolic content (A) and total flavonoid content (B) of different parts of pineapple

Antioxidant activities

Figure 2A shows MD2 pineapple peel had the highest DPPH scavenging activity compared with the flesh, mixture and core. These samples have relatively low scavenging activity are probably due to their low capability as H donor compared to pineapple peel. Besides, gallic acid standard had EC_{50} value of 33.00 ± 3.04 µg/ml. EC_{50} values (µg/ml) of various parts of MD2 pineapple are in descending order: core (54.68 ± 3.65) > mixture (45.00 ± 6.50) > flesh (41.50 ± 3.28) > peel (37.25 ± 2.70). The lowest EC_{50} value of pineapple peel showing the peel has the highest antioxidant activities. One-way ANOVA test revealed that there were significant differences between pineapple peel and other parts of MD2 pineapple, whereas the EC_{50} values of the flesh, mixture and core were not significantly differed. The variation in DPPH result compared to the total phenolics of different pineapple parts may be due to the other phytochemicals existed in the extracts. Also, climate change and use of pesticide or herbicide and the soil conditions of the pineapple planted may cause a variation in the phytochemical composition.[19]

Figure 2B shows the FRAP values (mM Fe^{2+}/g DW) of MD2 pineapple parts in descending order: peel (1.25 ± 0.248) > mixture (1.093 ± 0.53) > core (1.033 ± 0.53) > flesh (1.028 ± 0.10). The statistical results showed that there were significant differences in FRAP value between pineapple peel and the other parts of the pineapple ($p<0.05$), but FRAP value of pineapple flesh did not significantly different from the mixture and core. Moreover, some factors such as size and colour of a fruit and exposure of fruit to sunlight which may contribute to the variation in antioxidant activities of the fruit.[20]

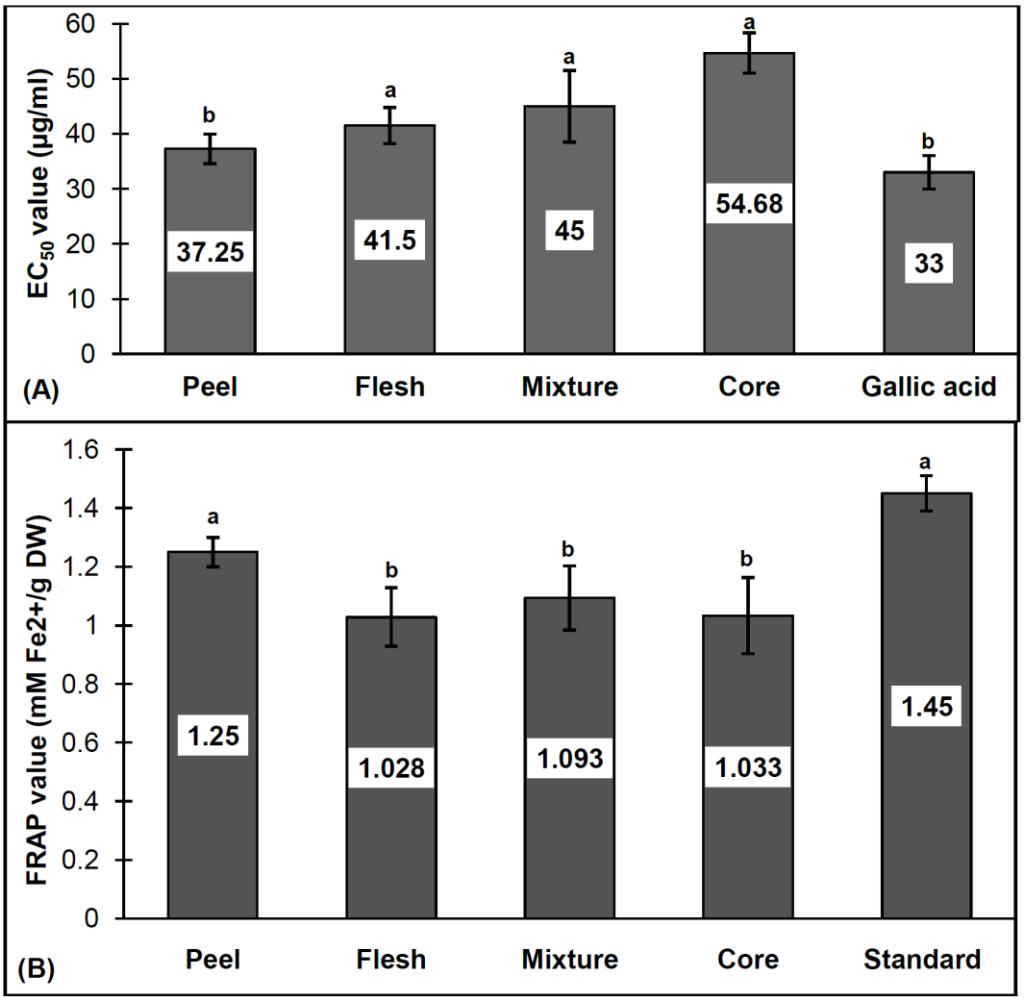

Figure 2 DPPH-EC_{50} value (A) and FRAP value (B) of various parts of pineapple

Correlation between total phenolics and antioxidant activities

As shown in Table 1, there are significant correlations between total phenolics and antioxidant activities of the pineapple samples. The results showed that there was a strong and negative correlation between DPPH assay and TPC ($R = -0.877$; $p<0.001$) and between DPPH assay and TFC ($R = -0.854$; $p<0.001$). Furthermore, there are strong and positive correlation between FRAP assay and TPC ($R = 0.643$; $p<0.01$) and between FRAP assy and TFC ($R = 0.796$; $p<0.01$). Antioxidant capacities as reported as in radical scavenging and

reduction of FRAP were correlated well with phenolic and flavonoid content, which could be noted that the strong antioxidant capacity of peel might be attributed to their high phenolic and flavonoid content in the extracts. This means that phenolic compounds are the main antioxidant components of MD2 pineapple identified by the DPPH and FRAP assays.[7]

Table 1 Correlations between total phenolics and antioxidant activities of MD2 pineapple samples

Antioxidant capacity	Total phenolic content		Total flavonoid content	
	r-value	p-value	r-value	p-value
DPPH assay (EC_{50} value)	-0.877**	0.000	-0.854**	0.000
FRAP value	0.643*	0.024	0.796**	0.002

** Correlation is significant at p<0.01; * Correlation is significant at p<0.05

Conclusion

This study is conducted to investigate total phenolics and antioxidant activities of different parts of MD2 pineapple, namely peel, flesh, core and mixture. The peel possessed the highest TPC and TFC compared with the other parts. Both TPC and TFC were significantly different between different parts of pineapple except the flesh and mixture. The peel had the highest antioxidant activities assessed by DPPH and FRAP assays. The samples that demonstrated the highest DPPH scavenging activity had the lowest EC_{50} values, and there were significant differences between gallic acid standard and all parts of pineapple. Therefore, different parts of MD2 pineapple are considered as new sources of nutraceutical especially the peel with high phenolics content and antioxidant activities should be fully utilised instead of being discarded.

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