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# Phytochemicals and Antioxidant Capacities from *Dacryodes rostrata* Fruits

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## Abstract

Nowadays, people are gaining interest on natural products due to the health conscious and awareness of the side effect caused by synthetic products. Malaysia is one of the top biodiversity, which host rich diversity of indigenous fruit, which could be a potential source of nutraceutical, antioxidant and pharmaceuticals. *Dacryodes rostrata* is an indigenous fruit found in Sarawak, Malaysia and is reported to be rich in oil, protein, minerals and could be a potential source of natural antioxidants. Thus, in this work, antioxidant components and antioxidant capacities of the extracts of peel, pulp and seeds of *D. rostrata* were evaluated. Total phenolic (1008 ± 24 mg/g GAE DW) and flavonoid contents (2551 ± 37 mg/g QE DW) of the seed extracts were higher when compared to peel and the pulp extracts. In addition, 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activity of the seed extracts were higher (63%), compared to peel (25%) and pulp (18%). Ferric reducing antioxidant power (FRAP) values of the seeds (1.25mM $FeSO_4$ equivalent) exhibited highest activity when, compared with peel (0.29 mM $FeSO_4$ equivalent) and pulp (0.90mM $FeSO_4$ equivalent). Seed extract also showed highest total antioxidant capacity determined by phosphomolybdenum method. Liquid chromatography-mass spectra (LC-MS) of the seed extracts from *D. rostrata* revealed the presence of phytochemicals in the form of gallic acid, ellagic acid, catechin, epicatechin and apigenin. Thus, *D.rostrata* seed extracts is having potent antioxidant capacity and could be used as a natural source of antioxidants.

**Keywords**: Antioxidant; *Dacryodes rostrata*; Extraction; Flavonoids; Phenolic compounds

## Introduction

Since ancient time, human have relied on mother nature for the basic needs including food, shelters, flavors, fragrances and also medicine [1]. Nowadays, people are getting more interested on the natural product remedies as they are more health conscious and aware of the side effects caused by synthetic product and drugs. Extensive studies and researches have been carried out on natural antioxidant and bioactive compounds in fruits, plants, seeds worldwide, due to the rising interest and market on the natural remedies and health supplement products [2].

Antioxidants were proven experimentally to be effective in lowering the oxidative stress and prevent the cellular damage caused by the free radicals generated in human body. Antioxidants have the ability to prevent and delay the onset of chronic diseases such as cardiovascular diseases, cancer, diabetes, stroke and dementia, which are caused by the oxidative stress [3,4]. However, the common synthetic antioxidants like butylatedhydroxytoulene (BHT), butylatedhydroxianisole (BHA) and tertiarybutylhydroquinone (TBHQ) have been reported to be toxic and carcinogenic to human body. This further lead to the exploration of natural, effective and economical antioxidant from natural sources such as fruits, vegetables and underutilized foods.

Malaysia is one of the top biodiversity spot in the world and has a rich diversity of indigenous fruits, that grow wildly in the peninsular Malaysia, Sabah and Sarawak. These indigenous fruits are the potential sources for the production of neutraceuticals, flavors and pharmaceuticals. However, these indigenous fruits are often underutilized due to their unknown features, and economical potential which have not been fully explored [4]. Several underutilized fruits are reported to be rich in bioactive compounds with prominent antioxidant capacity. For example, the oil obtained from the pulp and seed of an indigenous fruit, *Canarium odontophyllum* has shown to reduce oxidative stress and cholesterol level in animal models [5]. In addition, this fruit is also rich in phytochemicals like polyphenols which contribute to its antioxidant activity [6].

*Dacryodes rostrata* is one of the indigenous and underutilized fruit found in Sabah and Sarawak of Malaysia. The local communities commonly named it as kembayau. The fruit is ovoid to oblong in shape, dark purple in colour with a single seed in the center and is rich in proteins, minerals and oil. This fruit has been reported to possess significant nutritional content and rich in antioxidants in term of total phenolic and total flavonoid content [7]. This indicates that the extract from the *D. rostrata* has significant potential in developing into new herbal product or natural antioxidants. However, till date, this fruit is still underutilized and probably due to lacking of the information on its antioxidant activity as well as the phytochemicals that present in the fruit itself. Phytochemicals are bioactive compounds with antioxidant activity possessing various health benefits.

In this study, antioxidant activities of the *D. rostrata* fruits were evaluated. Different parts of the *D. rostrata* fruits, namely, peels, pulp and seeds were chosen in attempt to make a systematic comparisons among their antioxidant capacities and to identify the fraction with high antioxidant activity. In addition to that, the phytochemicals, which contribute to the antioxidant activity, were also been identified.

## Materials and Methods

### Chemicals and reagents

Methanol, hexane, hydrochloric acid, sulphuric acid (all analytical grades), acetonitrile and formic acid (HPLC grades) were purchased

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from Merch (Darmstadt, Germany) and ethanol (analytical grade) was obtained from Friendemann Schmidt (Parkwood, Australia). Gallic acid, sodium carbonate, Folin-Ciocalteu reagent, sodium nitrite, aluminum chloride, sodium hydroxide, quercetin, sodium phosphate, ammonium molybdate, Butylated hydroxytoluene (BHT), acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, ferrous sulphate, 1,1-diphenyl-2-picryl hydrazyl (DPPH) were purchased from Sigma (St. Louis, USA).

### Sample preparation

Fresh fruit of *D. rostrata* at mature stage was supplied by Agricultural Research Centre, Department of Agriculture, Sarawak, Malaysia. The fruits were transported to Monash University Malaysia in an icebox through airmail. The fruits without any physical damage with uniform shape and color were chosen and washed with tapped water and air-dried. The peel, pulp and seeds of the fruit were separated manually and freeze-dried. The freeze-dried samples were pulverized, sieved through 0.25mm sieve and stored at -20°C until further use.

### Ultrasonic extraction of sample for antioxidant assessment

Ultrasonic extraction was carried out in accordance to the method by Khoo et al. [6]. Before the extraction, the lyophilized sample was defatted using hexane and the sample was subjected for low temperature oven drying overnight to remove the solvent. The defatted sample (10g) was extracted with 500 ml of 50% ethanol with solid: solvent ratio of (1:50) using an ultrasonic cleaner (Thermoline, Thermo-6D, 40 kHz) at 30 °C for 30 minutes. The solution was then filtered using vacuum filter and the filtrate was subjected to freeze drying to remove the solvent. The freeze-dried extract was then stored at 4°C for further analysis.

### Determination of total phenolic content (TPC)

The total phenolic content was determined according to the method of Singleton and Rossi [8] and expressed as milligram gallic acid equivalent (GAE)/ 100 gram freeze-dried sample (FDS). A 100 µL aliquot of sample was added to 2 ml of 20 g/L sodium carbonate solution and incubated for 2 minutes. A 100 µL of 50% Folin-Ciocalteu reagent was then added and the solution was allowed to stand for 90 minutes at 25°C. The absorbance was measured at 750 nm against blank using a spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific). The blank consisted of all the reagents and solvents without the sample. A standard calibration curve of gallic acid with concentration ranging from 0.5 – 1000 mg/ml was plotted and the total phenolic content was determined using the standard gallic acid calibration curve.

### Determination of total flavonoid content (TFC)

Total flavonoid content was estimated following the aluminum chloride assay by Liu et al. [9]. In brief, 2 mL aliquot of sample was mixed with 0.2 mL of 5% sodium nitrite. After 5 minutes, 0.2 mL of 10% aluminum chloride was added to the mixture and mixed thoroughly. After 6 minutes, 2 mL of 1M sodium hydroxide was added and the mixture was made up to 5 mL using 50% ethanol and mixed thoroughly. The absorbance of mixture was read at 510 nm against blank using spectrophotometer. 50% ethanol was used as blank. A standard calibration curve of 0.5 – 1000 mg/ml quercetin was plotted and the total flavonoid content was calculated using the standard calibration curve and expressed as milligram quercetin equivalent (QE)/ gram freeze-dried sample (FDS).

### Determination of antioxidant capacity by phosphomolybdenum method

The antioxidant capacity was measured by the method of Prieto et al. [10]. Briefly, 0.1 mL of the sample was mixed with 1 mL of the reagent solution. The reagent which consisted of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture solution was then covered and incubated at 95 °C for 90 minutes. A blank sample containing all reagent and solvent without the sample was incubated under the same conditions. After the mixture was cooled down to room temperature, the absorbance was measured at 695 nm against the blank prepared earlier. A standard calibration curve of BHT was plotted to calculate the result and the antioxidant capacity was expressed as milligram BHT equivalent / gram freeze-dried sample (FDS).

### Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power was determined in accordance to the method by Kong et al. [7]. Three reagents including 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ were prepared. The FRAP reagent was prepared by mixing acetate buffer, TPTZ solution and $FeCl_3$ solution in proportion of 10:1:1 (v/v/v). An aliquot (50 µL) of the sample was mixed with 3 mL of the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance was measured at 593 nm against distilled water as blank. Ferrous sulphate (0 – 1000 µM) was used to construct calibration curve and the results were expressed as mmol $Fe^{2+}$/100 gram freeze-dried sample (FDS).

### Determination of 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals scavenging activities

The method of Prasad et al. [11] was adapted to determine the DPPH free radical scavenging activities of the extract. 0.1 mL sample was added into 0.9 mL of 200 µM DPPH solution dissolved in methanol and mixed thoroughly. The mixture was then incubated for 30 minutes at 28°C in dark and the absorbance was measured at 515 nm against blank. Methanol was used as blank and the control contained all the reagents without the samples. The antioxidant activity was expressed as percentage of scavenging activity of DPPH radicals and calculated using the following formula:

$$\textit{DPPH free radical scavenging activity, \%} = (A_0 - A_c)/A_0 \times \%$$

Where $A_0$ is the absorbance of control, $A_c$ is the absorbance of the sample.

### LC-MS analysis

The chromatography was performed on an Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer with dual ESI source using an Agilent Zorbax SB-C18 column (2.1 × 150 mm, 3.5 µm). Flow rate was 0.4 mL/min and the injection volume was 1 µL. Mobile phases consisted of 0.1% formic acid is Milli-Q water (A) and 100% Acetonitrile with 0.1% formic acid (B). The column was equilibrated for 5 min prior to each analysis. Separation was carried in 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 20 min, 95% B, 20.1 min, 100% B. The MS acquisition was performed in negative ionization information acquisition (IDA) between m/z 115 – 1100. The retention time and the mass obtained for the components were compared to the built in Metabolomics database.

## Results and Discussions

### Antioxidant capacities

From the experiment, the extract from kembayau seed exhibited a higher amount of both the TPC and TFC content (1007.96 mg GAE/g dw and 2550.90 mg QE/g dw respectively) followed by peel (560.28 mg GAE/g dw and 556.92 mg QE/g dw) and lastly pulp (273.03 mg

GAE/g dw and 287.24 mg QE/g dw respectively). The results were in accordance to the study done on kembayau and other underutilized fruits where the seed contained higher amount of phenolic and flavonoid followed by peel and pulp [7,12].

The antioxidant assays results were shown in Figures 1-3. From the DPPH radical scavenging activities result, the extract from seed exhibit highest scavenging activity and had superior antioxidant activity over the commercial synthetic antioxidant, BHT. Similar trends were observed in both FRAP and phosphomolybdenum analysis where the extract from seed exhibit the highest antioxidant capacity and is superior over both the BHT and ascorbic acid; the commercial antioxidants. The trends were in agreement with the study done by Kong et al. [7] and Guo et al. [13], who reported that the antioxidant capacities were higher in seed followed by peel and pulp for kembayau and other fruits.

## Phytochemicals

Phytochemicals are known to play an important role in prevention of many major diseases. Since, *D. rostrata* seeds exhibited high TPC, TFC and antioxidant activity, it was subjected to phytochemical analysis. From the LC-MS chromatogram of *D. rostrata* seeds, 9 polyphenols were tentatively identified (Table 1). Among them,

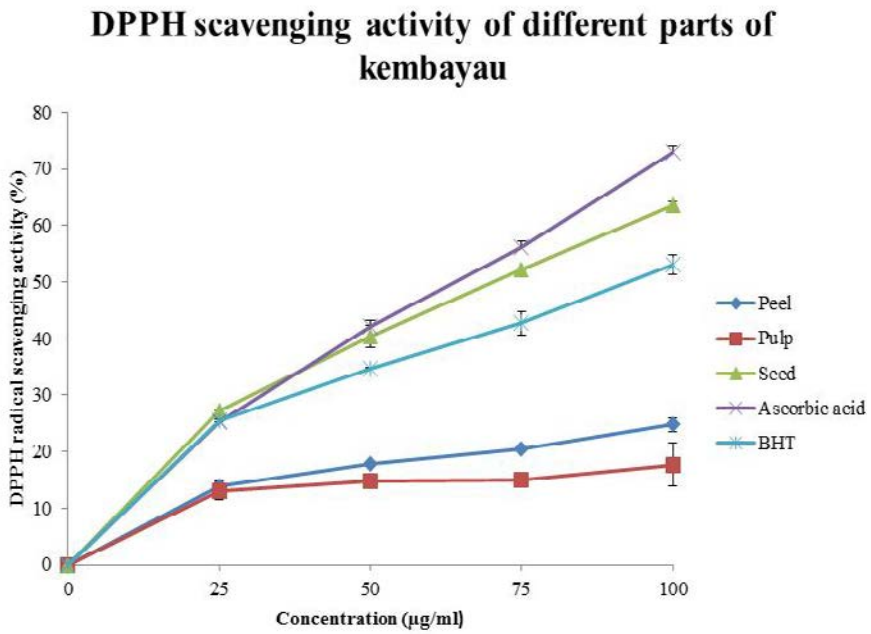


**Figure 1:** DPPH radical scavenging activities for different parts of kembayau, ascorbic acid and BHT.

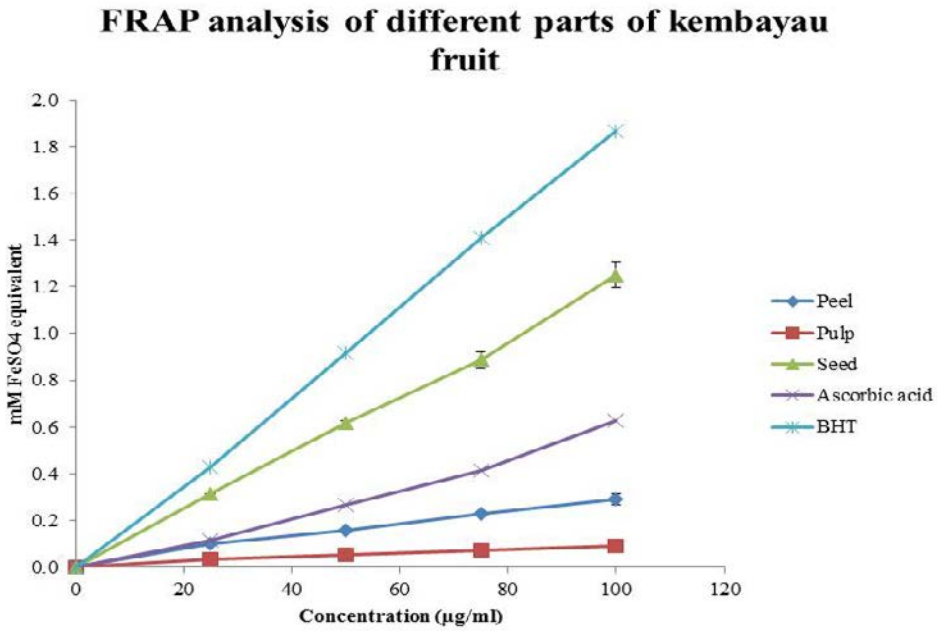


**Figure 2:** Ferric reducing antioxidant powers for different parts of kembayau, ascorbic acid and BHT.

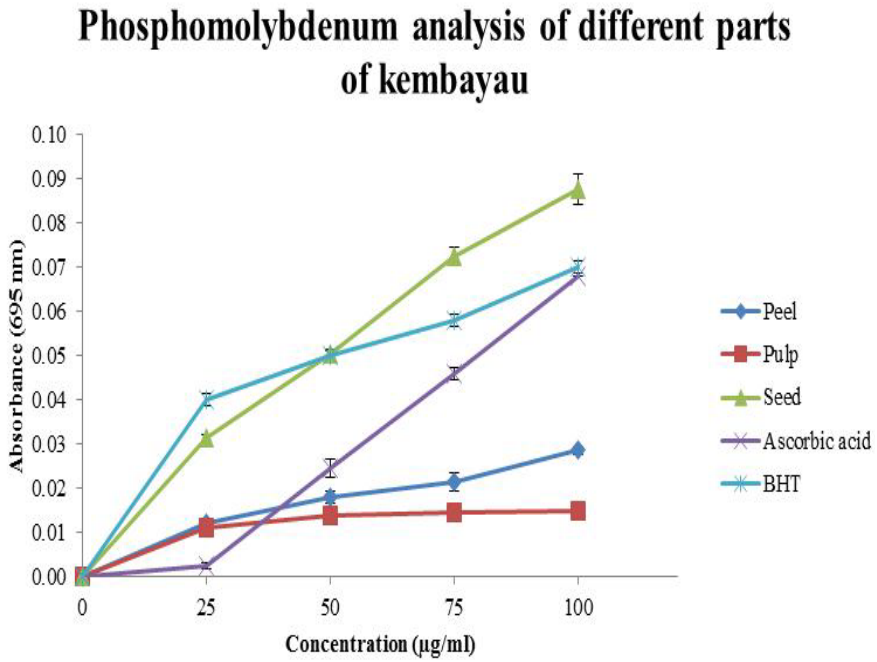


**Figure 3:** Antioxidant capacities by phosphomolybdenum method.

| No. | Compounds | Retention time (min) | m/z |
|---|---|---|---|
| **1** | Gallic acid | 2.052 | 169.014 |
| **2** | Epigallocatechin | 3.624 | 305.067 |
| **3** | Chlorogenic acid | 5.260 | 353.089 |
| **4** | Apigenin 7-(4''-Z-p-coumarylglucoside) | 5.020 | 577.136 |
| **5** | 1-Caffeoyl-4-deoxyquinic acid | 5.295 | 337.093 |
| **6** | 5-O-Caffeoylshikimic acid | 5.682 | 335.078 |
| **7** | (±)-Catechin | 5.682 | 289.073 |
| **8** | Syringic acid | 7.919 | 197.046 |
| **9** | Ellagic acid | 8.932 | 300.999 |
| **10** | (-)-Epicatechin 3-O-gallate | 9.439 | 441.083 |

**Table 1:** Phenolic compounds found in the seed of the kembayaufruitn and their chromatographic parameters.

chlorogenic acid and catechin were the major compounds along with minor compounds including gallic acid, ellagic acid among others. These phytochemicals are also reported from *Dacryodes edulis*. The presence of these phytochemcials confirmed the high antioxidant activity form *D. rostrata* seeds. The presence of these phytochemicals will be further verified by comparing the MS/MS fraction of the polyphenols standards.

## Conclusions

In conclusion, the seed of kembayau contained highest amount of flavonoids and phenolic compound as well as exhibited highest antioxidant capacities. The phytochemicals present in the kembayau fruits were tentatively identified as chlorogenic acid, catechin, gallic acid, ellagic acid, epicatechin and apigenin. The presence of the phytochemicals and the antioxidant capacities confirmed that *D. rostrata* seeds could be used potentially as a readily accessible source of natural antioxidant or as a potential source for pharmaceutical supplements. Future works on the extraction of phytochemicals from the kembayau seed are worth investigating.

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