Pharmacology and Clinical Pharmacy Research **ISSN:2527-7332 | e-ISSN: 2614-0020**
Volume 3 No 1 April 2018 **doi: 10.15416/pcpr.v3i1.16448**

Phaleria macrocarpa (Boerl.) Scheff Fruit: A Potential Source of Natural Antioxidant

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Abstract

Phaleria macrocarpa (Scheff.) Boerl is originated from Papua Island, Indonesia. There have been only limited attempts to explore the biological properties of this plant in relation to their medicinal use. This study aimed to examine antioxidant activity of P. *macrocarpa* fruit. Extraction of pericarp and mesocarp of *P. macrocarpa* were performed using soxhlet method with ethyl acetate as the solvent. Antioxidant activity was characterized in various *in vitro* model systems, including DPPH and ferric reducing antioxidant assay. We found that the highest amount of phenolic compounds and flavonoids were found in the pericarp (58.3±0.07 mg/g DW and 127.8±1.08 mg/g DW, respectively). The results showed that pericarp had higher antioxidant activity (IC_{50}= 122.4±1.14 µg/ml) compared to mesocarp (IC_{50}=175.48 ±1.75 µg/ml). In conclusion, the result of this study indicated the possible application of *P. macrocarpa* as a source of natural antioxidant compound.

Keywords: antioxidant, *Phaleria macrocarpa*, phenolic compounds, flavonoids

Introduction

Phaleria macrocarpa (Scheff.) Boerl is commonly known as crown of god or *mahkota dewa*. It is originated from Papua Island, Indonesia. This plant is popular in Indonesia. *P. macrocarpa* grows throughout the year in tropical area. Its fruit has eclipse shape with ø ± 3 cm. The colour of the fruit is green before ripening and red when fully ripe.[1] Traditionally, *P. macrocarpa* has been used to help reducing symptoms of cancer, impotency, hemorrhoids, diabetes mellitus, allergies, liver diseases, heart diseases, kidney diseases, acne, stroke, migraine, and various skin diseases.[2] Its fruit is usually consumed in the form of boiled water extract.

Despite the extensive use by Indonesian people, there have been only limited attempts to explore the biological properties of this plant in relation to their medicinal uses. This study aimed to examine antioxidant activity

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Received: 2 February 2018. **Revised:** 22 March 2018. **Published:** 23 April 2018.

of the extract of pericarp and mesocarp from P. *macrocarpa* fruit.

Methods

Extraction

The fruits of *P. macrocarpa* were obtained from Faculty of Mathematics and Natural Sciences, University of Riau, Pekanbaru, Riau, Indonesia. The mesocarp and pericarp were air-dried for 7 days. The extractions were performed using soxhlet. Briefly, air-dried powders of each part of *P. macrocarpa* fruit (0.5 g) were weighed and placed into a 100 ml conical flask. About 40 ml of ethyl acetate was added, followed by 10 ml and 6 M HCl solution. The mixture was magnetic stirred, placed in a sample flask (250 ml), and refluxed for 2 hours at 90º C. The mixture was filtered using Whatman No.1 and dried using vacuumed rotary evaporator at 40 ºC.[3]

Total phenolics assay

Total phenolic compounds were determined according to Ismail *et al*.[4] About 0.5 ml of each extract, 2.5 ml Folin-Ciocalteu reagent, 2 ml of 7.5% (w/v) Na_2CO_3 were vortex-mixed and incubated at room temperature for 90 minutes. The absorbances were measured using visible spectrophotometer (Novaspec II Visiblespectro) at 765 nm. The results were expressed as mg/g dry weight (DW).

Total flavonoid assay

The total flavonoid compounds in each extract was determined according to Ismail *et al*.[4] An aliquot (0.1 ml) of extract was added to 0.3 ml 5% (w/v) $NaNO_2$ and incubated for 5 minutes. About 0.3 ml 10% (w/v) $AlCl_3$ and 2 ml 1 N NaOH was added and the total volume was added up to 5 ml with distilled water. The absorbance was measured using visible spectrophotometer (Novaspec II Visiblespectro) at 510 nm. The results were expressed as mg/g DW.

Antioxidant activity

DPPH assay

The free radical scavenging activity of the extract was determined using the DPPH assay. Briefly, 1 ml extract was mixed with 3 ml 0.1 mM solution of 1,1-diphenyl-2-picryl-hydrazil (DPPH) in methanol. After incubation at room temperature for 30 minutes in dark condition, the absorbance of the mixture was mesured using a visible spectrophotometer (Novaspec II Visblespectro) at 517 nm. Ascorbic acid and α-tocopherol were used as positive controls. Free radical scavenging activity from the sample was calculated according to the following formula: $[(A_0 - A_1)/A_0] \times 100\%$; where A_0 was the absorbance of the control and A_1 was the absorbance of the sample.

Ferric-reducing antioxidant power (FRAP) assay

About 1 ml (concentration of 100, 150, 200, 250, and 300 µg/ml) of extracts were mixed with 2.5 ml of potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1 g/100 ml). The mixture was incubated at 50 ºC for 20 minutes. Trichloroacetic acid (10%) was added to the mixture to stop the reaction. Equal volume of distilled water was added followed by 0.5 ml ferric chloride (0.1g/100 ml) ($FeCl_3$). The analysis was conducted triplicate. The above procedures were repeated with BHT, ascorbic acid and α-tocopherol as positive controls. The percentage of antioxidant activity in FRAP assay of the samples was calculated according to the following formula: $(A_1-A_0)/A_1 \times 100\%$, where A_0=absorbance of the control (potassium phosphate buffer + FRAP reagent) and A_1=absorbance of the extract.

Results and Discussion

Phenolic and flavonoid contents

Phenolic is a class of secondary metabolites synthesized by plant which are utilized

Table 1. Total phenolic and flavonoid contents

Extract	Total phenolic (mg/g DW)	Total flavonoidb(mg/g DW)
Pericarp	58.3±0.07	127.8±1.08
Mesocarp	55.49±0.17	80.9±1.01

primarily for protection against stress. Several biological activities are associated with this compound, such as antioxidant, antimutagenic, anticarcinogenic, antiinflammatory and antimicrobial activities.[5-8]

As presented in Table 1, we found that the highest amount of phenolic compounds and flavonoids were found in pericarp (58.3±0.07 mg/ g DW) and 127.8±1.08 mg /g DW, respectively). In this study, the total flavonoid content was higher compared to the previous study which extracted *P. macrocarpa* dry fruit (without seed) using soxhlet with methanol as the solvent.

Antioxidant activity

Antioxidant is defined as a substance which significantly delays or inhibits oxidation process. Antioxidant activity is measured indirectly by determining the inhibition rate of oxidation process at the presence of antioxidant.[9] DPPH is widely used to determine the antioxidant activity of the plant extract.[10,11] The results showed that pericarp had higher antioxidant activity (IC_{50}=122.4±1.14 µg/ml) compared to mesocarp (IC_{50}=175.48 ±1.75 µg/ml).

The ability of extracts to reduce iron (III) to iron (II) was determined and compared to butylated hydrotoluene (BHT) which are known to be strong reducing agents. We found that the ability to reduce iron in the dose dependent manner in pericap and mesocarp were 91.66% and 53.28 %, respectively. This finding was slightly lower compared with previous study which used methanol as the solvent.[7]

The antioxidant activity of *P. macrocarpa* fruit might be due to the presence of phenolic and flavonoid compounds. Karimi et al,[12] reported the presence of kaempferol, myricetin, naringin, quercetin, and rutin as the major flavonoids present in *P. macrocarpa* fruit. The correlation between flavonoids and their antioxidant activity might be due to the presence of a 3-hydroxyl group in the heterocyclic ring while additional hydroxyl or methoxyl groups at positions 3,5 and 7 of rings A and C seem to be less important.[13-14] This finding was in accordance with previous study which investigated antioxidant activity of flavonoids.[15] Highly active flavonoids possess a 3'4'-dihydroxy occupied B ring and/or 3-OH group.

Conclusion

In conclusion, the result of this study indicated the possible application of *P. macrocarpa* as a source of natural antioxidant compound.

Table 2 . The IC_{50} values of the extracts

Extract	DPPH (µg/ml)	FRAP (µg/ml)
Pericarp	122.4±1.14	47.8±1.34
Mesocarp	175.48±1.75	71.2±1.24
BHT	78.7±1.01	24.8±1.32

Acknowledgements
The author would like to thank the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia for the laboratory facilities and Faculty of Mathematics and Natural Sciences, University of Riau for providing the samples.

Funding
None.

Conflict of Interest
None declared.

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