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Evaluation of DPPH free radical scavenging activity of *Pometia pinnata* from Indonesia

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Abstract
The antioxidant activity of *Pometia pinnata* which is widely used in traditional medicine has been analyzed. Extraction used solvent of methanol, ethyl acetate, and n-hexane. The research revealed DPPH free radical scavenging activity of *Pometia pinnata* extract varied widely increased with increase of concentration level. Among parts of *Pometia pinnata* fruit, its peel had higher antioxidant activity compared to its seed and flesh fruit. Antioxidant activity (IC_{50}) of peel with extracted methanol, ethyl acetate and n-hexane are respectively 1419 µg/ml, 917µg/ml, and 1195 µg/ml.

Keywords: Free radical scavenging, antioxidant activity, *Pometia pinnata*

1. Introduction
Oxidative stress is responsible for leading to various physiological and pathological abnormalities such as hypertension, inflammation, diabetes mellitus, atherosclerosis, aging and cardiovascular [1, 2]. It was caused by imbalanced of active oxygen species (free radicals) in the human body. Excess free radicals could cause damage to cellular protein, membrane lipid, and nucleic acid, and also cell apoptosis [2]. An antioxidant is required to protect their possible damages to biological molecules and to maintain an optimum balance of free radicals in human body. Epidemiological studies indicated that the antioxidant properties in fruits can use as protectors against certain diseases [3]. Fruits contained essential nutrients and secondary phytochemicals compounds [4]. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines,saponins, phenolics, flavonoids and glucosides [5]. In addition to fruits, their non-edible part was regarded as potential resources of antioxidant that have rich of polyphenol [6,7].
Indonesia has a large number of unexploited native and exotic fruit species, such as Matoa (*Pometia pinnata*). Many studies have determined the antioxidant capacities of *Pometia pinnata* fruits not only their byproduct but also the potential availability of antioxidants. Actually, it is important to measure bioavailability of antioxidant to know radical scavenging activity from part of Matoa (*Pometia pinnata*) fruit. Peel, seed, and flesh fruit have potentials of natural antioxidant properties. They are applicable in the pharmaceutical, cosmetic, food and feed industries, since they can be used as substitutes for synthetic antioxidant. Thus, the aim of this study was to measure antioxidant activity of peel, seed and flesh fruit of Matoa (*Pometia pinnata*) and to evaluate DPPH free radical scavenging activity.

Material and Methods
General experimental procedures
Pometia pinnata were obtained from local market in Pontianak, West Kalimantan. The flesh of *Pometia pinnata* have been dried in room temperature, and then made into powder. Reagent, methanol, ethyl acetate, n-hexane, and 1,1-Diphenyl-2-picrylhydrazil (DPPH) were purchased from Merck. All chemicals used were analytical grade

Sample extraction
Sample preparation was conducted by maceration using several organic solvents. About 150 g of each material such as powdered peel, seed, and flesh of Pometia pinnata, were immersed in 100 ml of n-hexane for 3 days, and then filtered. Filtrate was evaporated until dry sample was obtained, and this step resulted in raw extract of n-hexane. The residue from first immersion was entirely immersed back in 100 ml ethyl acetate for 3 days to obtain raw extract of ethyl

acetate. The solution was then filtered and filtrate was evaporated. The residue from this step was immersed in 100 ml methanol for 3 days, resulted in raw methanolic extract. The maceration process was repeated several times to obtain clear extract containing all of expected chemical species.

Antioxidant activity

Antioxidant activity of peel, seed, and flesh fruit of *Pometia pinnata* fruit extract was tested by monitoring radical scavenging activity using DPPH method [8]. Briefly, 1 ml of 200 µM DPPH (1,1-diphenyl-2-picrylhydrazil) solution in methanol was pipetted and transferred to vial. The samples were prepared separately to obtain 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml solution in methanol, and transferred to the vials which contain 200 µM DPPH. Each vial was diluted by adding methanol until the total volume of 5 ml. The absorbance of DPPH solution was measured by λUV-Vis spectrophotometer at 517 nm, every 5 minutes for total of 30 minutes. Antioxidant activity was calculated as a function of absorbance decrease of DPPH solution as a consequence of sample addition.

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ is absorbance of control and A_{sample} is the absorbance of the sample.

Result and Discussion

DPPH Free Radical Scavenging Activity of Methanolic Extracts of Part of *Pometia pinnata*

DPPH is nitrogen centered free radical having an odd electron which gives a strong absorption at 517 nm. Its color changes from purple to yellow when DPPH* odd electron paired off in the presence of radical scavenger to form the reduced DPPH-H [9, 10]. The DPPH assay results are indication of the hydrogendonating propensity of a test compound. Likewise, the antioxidant activity of plant extracts is also correlated with their reducing powers, which are generally associated with the presence of reductones. The DPPH radical scavenging activity is a good way to determine the antioxidant capacities in the sample [11, 12]. In present studies, DPPH free radical scavenging activity (%) of methanol extract of peel, seed and flesh fruit of Pometia pinnata fruit widely increased with the increasing of concentration level. Its linearity (R^2) was 0.672 to 0.949 (Table 1). Among parts of *Pometia pinnata*, DPPH free radical scavenging activity (%) of methanol extract of peel was the highest ranging from 30.08 to 47.60, followed by seed (27.66 to 35.79), and flesh fruit (12.22 to 15.73) at concentration 200 to 1000 µg/ml. The IC_{50} values of peel exhibited higher activity (1419 µg/ml) in comparison to both the other parts. On the other hand, flesh fruit which was extracted with methanol showed no antioxidant activity because IC_{50} value was very high. Another research showed the highest of DPPH radical scavenging activity in Matoa (*Pometia pinnata*) peel was possibly caused by secondary phytochemicals compounds, such as phenolic acid and flavonoid compounds [6, 7].

Table 1: DPPH Free Radical Scavenging Activity (%) of Methanolic Extracts of Part of *Pometia pinnata*

Part of *Pometia pinnata*	DPPH Free Radical Scavenging Activity (%) of Methanol Extract of *Pometia pinnata*						
	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml	Regression equal	IC-50 (µg/ml)
Peel	30.08	34.35	36.42	42.33	42.60	y = 0.016x + 27.29 R^2=0.949	1419
Seed	27.66	33.59	35.74	35.08	35.79	Y = 0.008x + 28.24 R^2=0.672	2720
Flesh Fruit	12.22	13.77	13.51	14.12	15.73	Y= 0.003x + 11.63 R^2=0.851	12790

DPPH Free Radical Scavenging Activity of Ethyl Acetate Extracts of Part of *Pometia pinnata*

In present studies, DPPH free radical scavenging activity (%) of ethyl acetate extract of peel, seed and flesh fruit of *Pometia pinnata* widely increased with the increasing of concentration level. Its linearity values (R^2) was 0.806 to 0.996 (Table 2). Among parts of *Pometia pinnata*, DPPH free radical scavenging activity (%) of peel was the highest ranging 32.38 to 52.08, followed by seed (27.18 to 30.44), and flesh fruit (29.27 to 32.23) at concentration 200 to 1000 µg/ml. The IC_{50} values of peel were exhibited higher activity (914 µg/ml) in comparison to seed (2688 µg/ml) and flesh fruit (7170 µg/ml). Another research showed the highest of DPPH radical scavenging activity in Matoa (*Pometia pinnata*) peel was possibly caused by secondary phytochemicals compounds, such as phenolic acid and flavonoid compounds [6, 7, 13]. The studi of Kanlayavattanakul *et al*. [3] reported that the IC_{50} values of DPPH scavenging radical activity on ethyl acetate fraction of the *Pometia pinnata* peel had high antioxidant activity compared with 70% ethanol fraction, and aqueous. The differences of IC_{50} values of DPPH scavenging radical activity might be caused by the differences solvent used. Besides, varieties of *Pometia pinnata* or differences part of fruit can also affect the value.

Table 2: DPPH Free Radical Scavenging Activity (%) of Ethyl Acetate Extracts of Part of *Pometia pinnata*

Part of *Pometia pinnata*	DPPH Free Radical Scavenging Activity (%) of Ethyl acetate of *Pometia pinnata*						
	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml	Regression equal	IC_{50} (µg/ml)
Peel	32.38	37.86	43.43	47.68	52.08	Y = 0.024x + 27.97 R^2=0.996	917
Seed	27.18	29.27	29.33	30.34	30.44	Y = 0.008x + 28.49 R^2=0.806	2688
Flesh Fruit	29.27	29.87	30.96	31.56	32.23	Y = 0.003x + 28.49 R^2=0.990	7170

DPPH Free Radical Scavenging Activity Of n-Hexane Extracts of Part of *Pometia pinnata*

DPPH free radical scavenging activity (%) of n-hexane extract of peel, seed and flesh fruit of *Pometia pinnata* widely increased with the increasing of concentration level. Its linearity values (R^2) was 0.837 to 0.999 (Table 3). Among parts of *Pometia pinnata*, DPPH free radical scavenging activity (%) of peel was the highest ranging 26.20 to 41.76, followed by seed (28.64 to 36.46), and flesh fruit (22.88 to 35.70) at concentration 200 to 1000 µg/ml.

The IC_{50} values of peel, seed and flesh fruit which extracted n-hexane solvent widely had low antioxidant activity (1195 µg/ml to 7656 µg/ml), because n-hexane was non polar solvent. The extraction of antioxidant substance of different chemical structure was achieved using solvent of different polarity [14]. Numerous investigations of qualitative composition of plant extracts revealed the presence of high concentration of phenolic in the extract obtained using polar solvent [15].

The extraction of peel, seed and flesh fruit of *Pometia pinnata* which was using different solvent, resulted the graphics of increasing of DPPH free radical scavenging activity. Graphical representative of DPPH free radical scavenging activity (% inhibition) of methanol, ethyl acetate and n-hexane of part of *Pometia pinnata* at different concentration levels is given by Figure 1

Table 3: DPPH Free Radical Scavenging Activity (%) of n-Hexane Extracts of Part of *Pometia pinnata*

Part of *Pometia pinnata*	DPPH Free Radical Scavenging Activity (%) of n-Hexan of *Pometia pinnata*						
	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml	Regression equal	IC_{50} (µg/ml)
peel	26.20	30.01	34.2	38.03	41.76	Y = 0.019x + 27.29 R^2=0.999	1195
Seed	28.64	33.54	34.11	34.3	36.46	Y = 0.003x + 27.03 R^2=0.837	7656
Flesh Fruit	22.88	23.3	24.41	24.54	35.70	Y = 0.015x + 17.60 R^2=0.851	2166

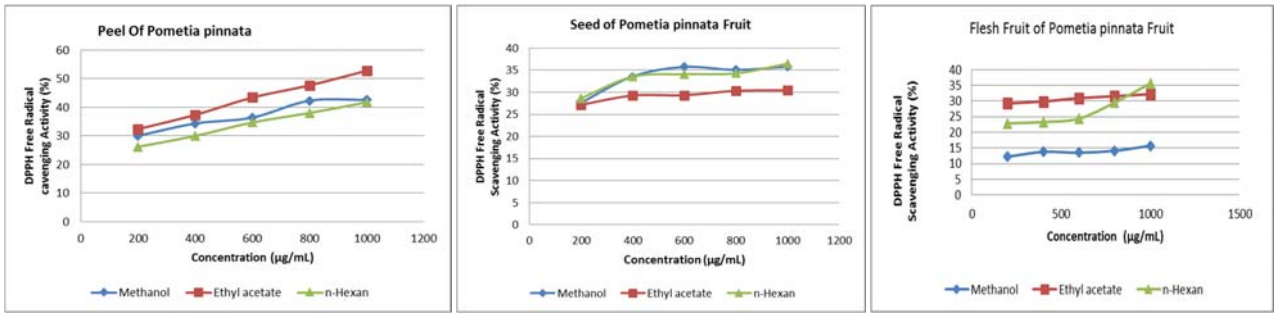

Fig 1: DPPH Free Radical Scavenging Activity of Methanol, ethyl acetae and n-hexane Extract of Peel, Seed and Flesh fruit of *Pometia pinnata*

Conclusion

This study revealed that DPPH free radical scavenging activity of peel, seed, and flesh fruit of *Pometia pinnata* which extracted with methanol, ethyl acetate, and n-hexan widely increased with the increasing of concentration level. Among parts of *Pometia pinnata*, its peel had higher antioxidant activity compared to seed and flesh fruit. Antioxidant activity (IC_{50}) of peel which extracted with methanol, ethyl acetate and n-hexane respectively 1419 µg/ml, 917 µg/ml, and 1195 µg/ml.

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