

The Potential of Java Plum (*Syzygium cumini*) as Source of Food Natural Antioxidant

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ABSTRACT

Research on Potential of Java Plum (Syzygium cumini) as Source of Food Natural Antioxidant was divided into 3 steps. The first was to prove that the fruit contain anthocyanin, with anthocyanin-betacyanin testing. The second was to compare antioxidant activity from 3 different stages of maturity of the fruit and with BHT. The third was to separate and identify pigments in the fruit extract.

First, the fruit was separated from the seed, freeze dried and kept in freezer during the research. Then, freeze dried fruit was extracted (macerated) with methanol-HCl 1% for overnight at 4 °C, and filtered with whatman no 1. This fruit extract was used for all steps of the research, sometimes concentrated with rotary evaporator at 40 °C when necessary.

The first step proved that the fruit extract contained anthocyanin. It was showed by the stable of pigment in HCl 2 M-100 °C for 5 minutes; alteration to green in addition of drops of 2 M NaOH; low to middle Rf value on HCl 1 %; and middle Rf value on BAW.

Antioxidant activity was tested with ferri-thiocyanate method with linoleic acid emulsion system. The results showed that the antioxidant activity of the fruit was influenced by maturity. Ripe (purple) fruit had highest antioxidant activity compared to unripe (red) and young (green) fruit. The antioxidant activity of purple, red, and green fruit were 64.75%, 62.42%, and 29.86% respectively; whereas BHT was 79.45%.

Separation of fruit extract on column chromatography with silica gel 60 as stationer phase and EtOAc to methanol: water (1:1) as mobile phase showed 3 different pigments: yellow, purple, and red respectively; whereas separation on paper chromatography with BAW showed 3 different spots: red, blue, and purple.

It could be concluded that Syzygium cumini contained anthocyanin. The fruit extract had antioxidant activity that influenced by stages of maturity. Ripe fruit had higher antioxidant activity than unripe and young fruit. At least there were 3 kinds of anthocyanin found in the fruit extract.

Key words: Syzygium cumini, anthocyanin, antioxidant activity.

INTRODUCTION

Java plum is an Indonesian tropical fruit that has local name 'duwet', 'jamblang', or 'jambolan'. The taste is sweetish-sour and a little bit astringent, the color of the ripe fruit is deep purple or blackish which often staining mouth that make children like it. They usually eat this fruit by it into a bowl, spread salt over them, then mix them together. The tree is usually grow in home garden or wild in field, and the fruit is usually sold in traditional market.

The fruit may be considered as a source of natural antioxidant because of the purple pigment, anthocyanins. These pigments are usually give bright color to many fruits, vegetables, and flowers, eg : red, purple,

and blue. In the previous time, these pigments were only known to attract insect that useful for seed dispersal. Recently, many researches that has been done on antioxidant activity of subtropical fruits showed that purple fruits had high antioxidant activity, like grapes, blue berries, and cranberries (Prior dkk, 2000). It seems that there is a correlation between anthocyanin content and antioxidant activity.

Anthocyanins are part of the very large and widespread group of plant constituents known collectively as flavonoids. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylum salts. The differences between individual anthocyanins are the number of hydroxyl groups in the molecule; the degree of methylation of these hydroxyl groups; the nature, number, and location of sugars attached to the molecule. The most frequently aglycone found in plants are: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Mazza in Aruoma et al, 1997).

Most fruits contain a mixture of anthocyanins from a simple pattern of only one pigment as in passion fruit or two anthocyanins as in peaches and pears, to a complex pattern of over twenty pigments as found in some grapes. In fruits the variation is by far more limited than in flowers. Cyanidin represents 55 %, Peonidin and delphinidin 12 % each, pelargonidin and malvidin 8 % each, and petunidin 6 %. The most common pigment is cyanidin-3 glucoside. Cyanidin is considered to be the most primitive pigment; in the advanced plants pelargonidin and delphinidin are more widespread (Gross, 1987).

Anthocyanins have different characteristics with another purple pigment, betacyanins, although the appearance in plant are similar. Both of them are polar and dissolved well in methanol-HCl 1%, but they never exist together in the same plant. Betacyanins are usually found in Centrospermae. There are several different characteristics that can be used to distinguish anthocyanin and betacyanin (Harborne, 1984).

In Indonesia there are many purple fruits and vegetables that might contain high anthocyanin and high antioxidant activity like: java plum, mulberry, passion

fruit, and purple-eggplant. Among these fruits, java plum has the deepest purple color from the skin to the seed surface; that is very easy to be extracted, even staining many things. Not many researches has been done on this fruit, maybe because of the poor economical value. Therefore research on this fruit will be very useful and interesting because of the great potential as source of antioxidant. Objectives of this research were to (1) Determine purple pigment in java plum fruit, whether it is anthocyanin or other red-purple pigment, betacyanin. (2) Measure antioxidant activity of the fruit extracts from 3 different stages of maturity, and with BHT. (3) Separation of pigments from fruit extract in methanol-HCl 1% with column chromatography and paper chromatography.

MATERIALS AND METHODS

Fruit Preparation and Extraction:

Fruit preparation and extraction is presented in Fig 1.

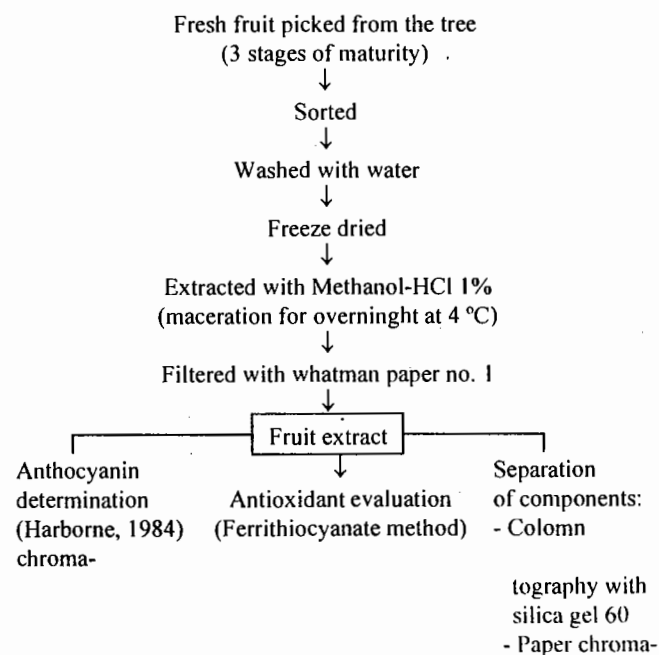


Figure 1: Fruit Preparation and Extraction

Anthocyanin determination

A special test was used to prove that the purple pigment in the fruit was anthocyanin, and not another d-purple pigment, betacyanin. The test is shown in table 1.

Table 1. Testing to distinguish Anthocyanin with other purple-red pigment

Testing	Anthocyanin	Betacyanin
Heating with 2 M HCl, 100 C for 5 minutes	Stable Red	Red →Colorless
Addition with 2 M NaOH by drops	Red →Blue green	Red →Yellow
Chromatography with HCl 1 %	Rf low to medium	Rf high
Chromatography with BAW	Rf medium (10-40)	Rf very low (00-10)
Maximum absorbance in metanol-HCl	505-535 nm	532-554

Antioxidant Activity

Antioxidant activity was evaluated by thiocyanate method with linoleic acid-emulsion as substrate. The incubation temperature was at 30 °C, and the absorbance of samples and control (without fruit extract) were measured at 520 nm every 24 hours for 6 days (On 7th day absorbance of control was decreased, indicated that the measurement had finished).

Pigment Separation

Column Chromatography

Pigment separation was done with column chromatography (d=1.5 cm; l=30 cm) with silica gel 60 as a stationary phase and EtOAc with gradient elution to ethanol/ water (1:1) as mobile phase. Elution was done gradually with combination of solvent A (EtOAc) and solvent B (methanol : water = 1:1), with composition : 100% A, 75 % A/B, 50 % A/B, 25 % A/B, 100% B.

Volume for each combination of solvents was 50 ml (Smith dkk, 2000).

Crude extract of 3 g of freeze dried fruit with methanol-1 % HCl, was then evaporated to get small volume (2 mL) that was applied to the column, followed by the developing solvents.

Paper chromatography

The second way to separate the pigment of fruit extracted was paper chromatography, (Whatman no. 1) with three developing solvents commonly used for anthocyanin identification (Harborne 1984). They are listed below:

Developing solvent 1: BAW (Butanol : HOAc : H₂O = 4:1:5)

Developing solvent 2: Bu-HCl (Butanol:HCl 2M = 1:1)

Developing solvent 3: HCl 1%

4. RESULTS AND DISCUSSIONS

The fruit were in 3 different stages of maturity.

Stage 1 (Green, young) : The color of the fruit was green, the texture was hard.

Stage 2 (Red, unripe) : The color of the fruit was red, the texture was hard.

Stage 3 (Purple/ Black, ripe) : The color of the fruit was purple to black, the texture was soft.

The fruits from 3 different stages of maturity were extracted with methanol-1% HCl, and be used for evaluation of antioxidant activity; the results showed that the highest was the ripe fruit. Therefore only the ripe fruit was used for anthocyanin determination and pigment separation with both column chromatography and paper chromatography.

Anthocyanin Determination

Anthocyanin determination of the ripe-fruit extract was showed that the fruit extract contained anthocyanin, and not other purple pigment betacyanin (Table 2).

Table 2. Anthocyanin Determination on Java Plum Fruit Extract

No.	Testing	Anthocyanin
1.	Heating with 2 M HCl, 100 °C for 5 minutes	Stable Red
2.	Addition with 2 M NaOH by drops	Red →green
3.	Paper Chromatography with HCl 1 % as developing solvent	Rf : 13 - 31 (low to medium)
4.	Paper Chromatography with BAW as developing solvent	Rf: 31 – 43,5 (medium: 10-40)
5.	Maximum absorbance in metanol-HCl	510-530 nm (505-535 nm)

All of the results fulfilled the characteristics of anthocyanins: the fruit extract was red in methanol-1%HCl, and the red color was stable when heated with 2 M HCl 100 °C for 5 minutes; alteration of red color to bluish-green when added with 2 M NaOH by drops; Rf = 13-31 (the requirement was low to medium) in paper chromatography with 1 % HCl as developing solvent; Rf = 31-43,5 (the requirement was medium : 10-40) in paper chromatography with BAW as developing solvent; visible spectrum in methanol-HCl : maxi-

imum absorbance was at 535 nm (the requirement was 505-535 nm).

Antioxidant Activity

Antioxidant activity evaluation showed that the fruit extracts from different stages of maturity have different antioxidant activity. The antioxidant activity was influenced by stages of maturity, purple-ripe fruit had the highest antioxidant activity than red and green fruit, red fruit had higher antioxidant activity than green fruit (Table 3).

Calculation of Antioxidant activity was based on increasing of samples absorbance compared to increasing of control absorbance :

Antioxidant activity: $100 - \left\{ \frac{\text{Increasing of A sample}}{\text{Increasing of A control}} \times 100 \right\} \%$

Separation of Fruit Extract Components

Column Chromatography

Separation of the ripe fruit extract on column chromatography showed that there were 3 pigments. The pigments came out from the column as listed below :

- First : Yellow (± 100 mL)
- Second: Purple (± 10 mL)
- Third: Red (± 50 mL)

Table 3. Antioxidant Activity of the Extract from Java Plum at Different Stages of Maturity

No.	Absorbance at $\lambda = 520$						Antioxidant Activity
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	
Green	0,153	0,225	0,316	0,407	0,558	0,874	29.86 %
Red	0,150	0,176	0,234	0,291	0,354	0,526	62.42 %
Purple	0,143	0,181	0,250	0,369	0,393	0,505	64.75 %
BHT	0,132	0,114	0,124	0,154	0,216	0,343	79.45 %
Control	0,172	0,315	0,501	0,706	1,122	1,199	

*) average from duplicate analysis

It can be concluded that the yellow pigment was more non polar than the purple and red pigments, and the red pigment was the most polar compared to the yellow and purple pigments.

Paper Chromatography

The result of ripe-java plum fruit extract separation on paper chromatography with 3 developing solvents commonly used for anthocyanin identification could be seen at table 4.

Tabel 4. Separation of Java Plum Fruit Extract on Paper Chromatography

Eluent	Spot	Possibility
BAW	Rf = 40	Pelargonidin 3-(p-coumarylglucoside)-5-glucoside
	Rf = 31	Pelargonidin 3,5-diglucoside
	Rf = 25	Cyanidin 3-rhamnosylglucoside-5-glucoside
Bu-HCl	Rf = 14	Pelargonidin 3,5-diglucoside
	Rf = 8	Cyanidin 3-rhamnosylglucoside-5-glucoside
HCl 1 %	Rf = 36	Cyanidin 3-rhamnosylglucoside-5-glucoside
	Rf = 23	Pelargonidin 3,5-diglucoside

BAW as developing solvent resulted 3 spots with Rf = 40; 31; and 25. Bu-HCl as developing solvent resulted 2 spots with Rf = 14 and 8. HCl 1 % as developing solvent resulted 2 spots with Rf = 36 and 23. Each spot indicated an anthocyanin, and the Rf for each developing solvent is specific for a certain anthocyanin (Harborne, 1984).

The data showed that BAW could separate the fruit extract into 3 anthocyanins: Pelargonidin 3-(p-coumarylglucoside)-5-glucoside (Rf=40); Pelargonidin 3,5-diglucoside (Rf=31); and Cyanidin 3-rhamnosylglucoside-5-glucoside (Rf=25). Bu-HCl could separate the fruit extract into 2 anthocyanins: Pelargonidin 3,5-diglucoside (Rf=14); and Cyanidin 3-rhamnosylglucoside-5-glucoside (Rf=8). HCl 1% could separate the fruit extract into 2 anthocyanins: Cyanidin

3-rhamnosylglucoside-5-glucoside (Rf =36); and Pelargonidin 3,5-diglucoside (Rf=23).

The Rf values of the 2 spots that were found in three developing solvents, were appropriate each other to indicate that they were: Cyanidin 3-rhamnosylglucoside-5-glucoside and Pelargonidin 3,5-diglucoside. Another spot that could only be found in BAW, and could not be found in Bu-HCl and in HCl 1 % was Pelargonidin 3-(p-coumarylglucoside)-5-glucoside. It showed that this anthocyanin could only be separated with BAW, and was known as acylated anthocyanin, a stable form of anthocyanin.

Based on the data resulted from separation of fruit extract on paper chromatography (Rfs from BAW, Bu-HCl, HCl 1 %) and according to Harborne (1984), it could be concluded that the ripe java plum extract contained 3 different anthocyanins: Pelargonidin 3,5-diglucoside; Cyanidin 3-rhamnosylglucoside-5-glucoside; and Pelargonidin 3-(p-coumarylglucoside)-5-glucoside.

CONCLUSIONS:

From the results of the research, it can be concluded Java plum (*Syzygium cumini*) fruit, especially the ripe-fruit contained anthocyanin, and not other red-purple pigment, betacyanin. The antioxidant activity of java plum fruit was influenced by stages of maturity, the purple-ripe fruit had the highest antioxidant activity, followed by the red-unripe fruit; and the lowest was the green fruit. Their antioxidant activity were: 64.75 %; 62.42 %; and 29.86 % for ripe, unripe, and green fruit respectively; whereas BHT was 79.45 %. Apparently, anthocyanin played a role in this phenomena, because the red and purple color developed with the maturation process and the increasing of antioxidant activity. Separation with column chromatography with silica gel G-60 as stationer phase and gradient emobile phase of EtOAc to methanol: water (1:1) showed 3 pigments: 1 yellow pigment; 1 brownish-red pigment; and 1 red pigment. Separation with paper chromatography showed three different anthocyanins: Pelargonidin 3,5-diglucoside; Cyanidin 3-rhamnosylglucoside-5-glucoside;

side; and Pelargonidin 3-(p-coumarylglucoside)-5-glucoside.

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