Comparison of Vitamin, Anthocyanin, and Bioactive Compounds from Gajah and Padi Jengkol (\textit{Archidendron jiringa}) Peel as Potential Natural Antioxidants

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Abstract. Synthetic antioxidants have recently been reported to be dangerous for animal and human health. Thus, natural antioxidants from by-product plant materials are potential to replace synthetic ones. Vitamin C, E, carotenoid, anthocyanin, and phenolic compound have been proposed as a biological antioxidants. This experiment was designed to evaluate and compare the content of vitamin (C and E), anthocyanin, and bioactive compound (tannin and total phenol) from Gajah and Padi jengkol peel to assess their potentials as natural antioxidants. Data were analyzed descriptively by calculated the average of the data. The results showed that there were no differences between vitamin C and E percentage from gajah and padi Jengkol peel. Percentage of vitamin C was in low category and vitamin E was in medium category. Anthocyanin content of Padi Jengkol peel was 37.35\% higher than Gajah Jengkol peel. Likewise, tannin and total phenol content of padi jengkol peel were almost 2 times higher than the gajah jengkol peel. It is concluded that padi jengkol peel are more potential to be used as a natural antioxidants than gajah jengkol peel.

Key words: bioactive compound, jengkol peel, natural antioxidants, vitamin

1. Introduction
Free radicals are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals leads to oxidative stress. This oxidative stress causes damage to tissues and results in large number of diseases \cite{1}. The antioxidants are an important role in protection against disorder caused by oxidant damage. Halliwell \cite{2} reported that an antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. Recently there has been a considerable interest in finding natural antioxidants to replace synthetic antioxidants. Lobo \textit{et al.} \cite{3} reported that synthetic antioxidants have recently been reported to be dangerous for animal and human health.

Natural antioxidants occur in all higher plants and in all parts of the plant (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen, and seeds) \cite{4}. Jengkol (\textit{Archidendron jiringa}) is tropical plant that available in high quantity. There are two popular varieties of jengkol such as gajah and
Hidayah et al [5] reported that peel from jengkol plant is by-product had potential as a crude fiber, energy and saponin source which can be used as an alternative to natural feed additive to increase animal productivity. There are limited studies about vitamin content from gajah and padi jengkol peel that can use to predictable natural antioxidants alternatives. Vitamin C, E, carotenoid, anthocyanin, and phenolic compound have been proposed as a natural antioxidants [6]. Vitamin C (ascorbic acid) can reduce and neutralize free radical such as hydrogen peroxide [7]. Vitamin E (α-tocopherol) can protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [3]. Phenolic compound is reducing agent, hydrogen donating antioxidants, and oxygen quenchers. So base on that background, this study is aimed to evaluate and compare the content of vitamin (C and E), anthocyanin, and bioactive compound (tannin and total phenol) from gajah and padi jengkol peel to assess their potentials as natural antioxidants.

2. Materials and Methods

2.1 Preparing Materials

The gajah and padi jengkol peel was dried for 5-6 hours under the sun until got stable weigh. After that, the materials were milled with grinding machine to got materials into a fine powder form.

2.2 Determination of Vitamin C Content [8]

The HPLC analysis was carried out to determine the vitamin C on a Shimadzu class LC VP HPLC system with class LC-VP software, a pump (LC-6- AD), and a UV-VIS detector (Pack-ODS (250 mm x 4.6 mm I.D., 5 μm) for organic acids and SGE (250 mm x 4.6 mm I.D., 5 μm). The mobile phases were water adjusted to pH 3 with phosphoric acid (vitamin C). Separation was carried out by isocratic elution with a flow rate of 0.4 ml min-1 and column temperature was ambient. The UV detector was set at 210 nm and 254 nm, respectively. Quantitation was based on the peak area measurement. Sample (10 g) was extracted in 10 ml water adjusted to pH 1.5 with 10 ml phosphoric acid-water (2%, v/v). The extracts were filtered through filter paper. Then, 1.5 ml buffer (0.01 M KH2PO4 , pH 8.0) was added to 1.5 ml sample extract. From this, 1 ml of these mixtures were loaded on to C18 cartridges. After loading, 3 ml water adjusted to pH 1.5 and 2 ml phosphoric acid-water (2%, v/v) was passed through the cartridges. For HPLC, 20 μl of the eluents were injected.

2.3 Determination of Vitamin E Content [9]

The chromatographic analysis was carried out in a HPLC integrated system equipped with an AS-950 automated injector, a PU-980 pump, an MD-910 multiwavelength diode array detector (DAD) and an FP-920 fluorescence detector (Jasco, Japan). The chromatographic separation of the compounds was achieved on a normal phase SupelcosilTM LC-SI (3 mm) 75 3.0 mm (Supelco, Bellefonte, PA), operating at constant room temperature (21 8C). A mixture of n-hexane and 1, 4-dioxane (98 : 2) was used as eluent, at 0.7 mL/min. The detection was performed by both the DAD connected in series with the fluorescence detector, programmed for excitation at 290 and emission at 330 nm (gain 10).

2.4 Determination of Anthocyanin and Total Phenol Content [10]

Elution solvents used were: A, 10% (v/v) aqueous HAc; and B, acetonitrile. A flow rate of 1 ml min-1 was used with a linear 30 min gradient from 0 to 30% B followed by a 5 min hold at 30% B. The column was then washed with 50% B for 5 min, returned to 0% B and re-equilibrated for 10 min before the next analysis. The eluted components were monitored at 280-313 nm for the remaining phenolic acids and 530 nm for anthocyanin. Preparative HPLC was used to isolate individual anthocyanin and flavonoids. Samples (10-20 g) were extracted as above and partially-purified on a column of RP-18 before preparative HPLC on a 300x25 mm Rainin Microsorb RP-18 column (pore size with a 60 Ø) 110x25 mm guard column and a flow rate of 12 ml min^-1. The solvents, A and B, were the same as for the analytical HPLC. Tuber anthocyanin were separated using a linear solvent gradient from 8-10% B for 20 min, followed by a linear gradient from 10 to 20% B for 15 min and
monitored at 530 nm. Flower flavonoids were resolved with a linear solvent gradient from 0-30% B for 30 min and monitored at 350 nm.

2.5 Determination of Tannin Content [11]
500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M. The absorbance was measured at 120 nm within 10 min.

2.6 Data Analysis
Data were analyzed descriptively by calculated the average of the data.

3. Results and Discussions
The content of vitamin, anthocyanin, and bioactive compounds gajah and padi jengkol peel was showed in Table 1. There were no differences between vitamin C and E percentage from gajah and padi jengkol peel. Percentage of vitamin C was in low category and vitamin E was in medium category. Dumbrava et al.[10] stated that the source of vitamin C from tropical fruits and vegetables that contain high levels of ascorbic is around 984.80 mg/100 g. Ciftci et al. [11] reported that dietary supplementation by 200 mg vitamin C plus 125 mg vitamin E/kg of diet may increase egg production and improve egg quality in laying hens during heat stress. Vitamin C is popular with anti-oxidant defenses, not only by reacting with all oxygen species through formation of dehydroascorbyl, a particular inert radical, but also by transferring radical equivalents from lipid phases to aqueous compartment [2]. Vitamin C (ascorbic acid) can reduce and neutralize free radical such as hydrogen peroxide [7]. Vitamin E (α-tocopherol) can protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [3].

### Table 1. The Content of Vitamin, Anthocyanin, and Bioactive Compounds Jengkol Peel

| Jengkol Varieties | Vitamin C (mg/kg) | Vitamin E (mg/100g) | Anthocyanin (mg/100g) | Tannin (%) | Total Phenol (%) |
|-------------------|-------------------|----------------------|-----------------------|------------|-----------------|
| Gajah             | < 0.70            | 0.92                 | 104.44                | 5.73       | 8.98            |
| Padi              | < 0.70            | 0.91                 | 166.72                | 12.24      | 15.29           |

Anthocyanin content of padi jengkol peel (166.72 mg/100 g) was 37.35% higher than gajah jengkol peel (104.44 mg/100 g). Sass-Kiss et al. [12] reported that variety is an important factor affecting composition and content of pigment. Anthocyanin is reported to have some therapeutic benefits including vaso-protective and anti-inflammatory properties, anti-cancer and chemoprotective properties [13,14]. Anthocyanin contribute greatly to the antioxidant properties of certain colourful foods [15]. Anthocyanin content in this research higher than anthocyanin of black sorghum (40-98 mg/100 g) that believed superior brans as a source of antioxidants [16].

Likewise, tannin and total phenol content of padi jengkol peel were almost 2 times higher than the gajah jengkol peel. The different result reported Hidayah et al. [5] that gajah jengkol peel had higher tannin and total phenol content than padi jengkol peel. Preface [17] stated that the different result total phenol and tannin content are caused by stage of maturity of the plant and chemo-type, agronomic factors (e.g. conditions of cultivation and harvesting, irrigation, fertilization and methods of harvest). According to Kliebenstein [18] the different level of phenolics in plants is affected by many factors including genetic and physiological factors as well as environmental factors (high and low temperature, drought, alkalinity, salinity, UV stress, bacteria, fungi, insects, etc).

Tannins are phenolic secondary compounds of plants that principal function is to provide protection against microbial pathogens, insect, pests, and herbivores [19]. Phenolic compound is reducing agent, hydrogen donating antioxidants, and oxygen quenchers [3]. Vasta et al. [20] reported that supplementation of *Cistus ladanifer* on lamb meat had a protective effect against oxidation and
reduced lipid autoxidation on lamb. The same result reported by Luciano et al. [21] inclusion of a polyphenol-rich extract from quebracho on diets can delay myoglobin oxidation and extend the color stability of meat stored both in high oxygen modified atmosphere and in aerobic conditions.

4. Conclusion
Based on vitamin C, E, anthocyanin, tannin and total phenol content, it can be concluded that padi jengkol peel more potential as a natural antioxidants than gajah jengkol peel.

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6. References
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