Flavonoid of some antioxidant plants in Taman Wisata Alam Pangandaran

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Abstract. Flavonoids can be found in every organ of plants and has a role as natural antioxidant. The aim of this research was to investigate the influence of maceration and drying time to the level and the type of flavonoids from some leaves of plants located in Taman Wisata Alam Pangandaran. This research used CRD (Completely Randomized Design) with 2 factorial design. The first factor was soaking time of the plant extract in ethanol 96% for 24 hours and 48 hours, the second was drying time for 14 and 21 days. Sample used were Bungur (Largerstoremia spectosa L.), Mahoni (Swietenia macrophylla), Nyamplung (Calophyllum inophyllum L.), dan Vitex (Vitex pubescens Vahl.) leaves. Qualitative parameter was determined from the change of color of the solution as well quantitatively by spectrophotometer of 415nm. Flavonoid type was then analysed using spectrophotometer at 250-560nm. Qualitative result showed that all plants assessed contain flavonoid in varying level. The type was also ranging between each sample. Quantitative analysis of 2 ways ANOVA showed that maceration and drying were shown significance influence with optimum flavonoid level varying from one plant to another. It can be concluded that all plants tested positively contain flavonoid with different type and immersion as well as drying has significantly affected the quantity of flavonoid result in plants.

1. Introduction
Indonesia is tropical countries, having biodiversity second largest in the world after Brazil. The natural resources herbs in Indonesia consists of 30,000 sorts of crops of the total 40,000 sorts of crops in the world, where 940 types of them were herbs efficacious a drug (this number is 90 % of the number of herbs drugs in Asia) [1].

Free radicals might be produced from body metabolism product, cigarette smoke, air pollution, certain drugs, ultraviolet light, and radiation [2]. Free radicals involved in such degenerative diseases as pathogenesis of diabetes, liver damage, inflammatory, cancer, a heart problem, a nervous disorder and aging process [3]. Therefore, antioxidants are needed to help the body from free radicals’ attack and reduce the negatives impact of it [4].

Human beings have endogenous antioxidant from their body, since free radicals’ molecules are growing up, endogenous antioxidants to banish the damage on the cells the body are direly needed [5]. Hence, the body needed the fillings of exogenous antioxidant originated from outside of the body (obtained in nature) and help to reduce the negative impact of an oxidant and free radical [6,7].
Compound antioxidant can be found in two types, namely natural and synthetic antioxidant. Natural antioxidant is chosen as the additional antioxidant than the synthetic one, because carcinogenic effect of synthetic may develop [8]. Plants have been decades known as major source of natural antioxidants due to their phytochemical contain [9]. Phenolic compound found in plant is responsible reason of antioxidant property, which the greater phenol compound content, the greater also the antioxidant activities in plant [10,11]. One of the most common antioxidant compounds that found in plant tissues is flavonoid [12]. Some endemic plants even have greater flavonoid content than others. One example is Manggong Bamboo leaves which is almost as strong as vitamin C due to its active compound found (flavonoid, triterpenoid, alkaloid and saponin) [6]. Other research investigated strong antioxidant property from some fruit plant such as mangosteen, citrus, banana and many more fruits [13-15].

Flavonoid can be found in every organ of plant, so it will be found on each plant extracts [16]. Flavonoid is the group of polyphenol compound known to able to against free radicals (oxygen reactive compound) such as superoxide anion and radical hydroxyl, inhibitors for enzyme hydrolysis and oxidative, and worked as anti-inflammation [17].

Flavonoid is secondary metabolite compound which is found in plant, except algae. Common flavonoids found in higher plants (angiosperm) are flavone and flavanol content with C- and O-glycoside, isoflavones C- and O-glycosides, flavanones C- and O-glycosides, chalcones with C- and O-glycosides, and dihydrochalcones, proanthocyanins and anthocyanin, aurores O-glycosides, and dihydroflavonols O-glycoside. Flavones, flavanols, flavanones, isoflavones, and chalcones also often found in the form of glycol. Flavonoid is constituted of two aromatic rings that able or unable to form the third ring with the arrangement of C6-C3-C6 [16].

Taman Wisata Alam Pangandaran (TWA) has some sorts of plants which are believed to be a natural antioxidant, they are Bungur (Largerstoremia speciosa L.), Mahoni (Swietenia macrophylla), Nyamplung (Calophyllum inophyllum L.), and Vitex (Vitex pubescens Vahl.). Flavonoid content of these plants may vary each other. Therefore, the study was done to investigate the type and quantity of flavonoid in the plants mentioned.

2. Method

This research used CRD (Completely Randomized Design) with 2 factorial experimental designs. The first factorial was ethanol (96%) soaking duration (24 and 48 hours), the second was draining period of leaves (14 and 21 days). Sample used were leaves of vitex, mahoni, nyamplung, and bungur which obtained from TWA Pangandaran, West Java. Experimental design was shown in table 1. Samples were group based on the table as P1M1 (14 days drain and 24 hours maceration), P2M1 (21 days drain and 24 hours maceration), P2M1 (14 days drain and 48 hours maceration) and P2M2 (21 days drain and 48 hours maceration).

| Maceration | Drain |
|------------|-------|
| 24 hours (C) | P1M1 | P2M1 |
| 48 hours (D) | P2M1 | P2M2 |

The leaves of vitex, bungur, nyamplung, and mahoni were collected from TWA Pangandaran then dried with windy-drying techniques. According to Lumbessy, this stage begun by cutting and mashing sample leaves of to make surface smaller, next maceration was done in the tube reaction using ethanol 96% for every test [18]. Maceration was done for 24 hours and 48 hours in any variation drying. The qualitative test was done to ensure the flavonoid content. Filtrate was inserted (±1 ml) into test tube, 20 ml hot water was added, then boiled for 5 minutes. Another 0,5 gram and 10 drops of HCl were mixed in, color formed was then observed (red or in pink or yellow show positive flavonoid). Types of flavonoid was
also determined by analyzing their absorbance within UV-Vis spectrum. Bands formed was indicated the type of flavonoid in each plant (Table 2).

**Table 2. Range of Flavonoid UV-Vis Absorption Spectrum [16].**

| Band II (nm) | Band I (nm) | Kind of flavonoids                              |
|--------------|-------------|-------------------------------------------------|
| 250-280      | 310-350     | Flavone                                         |
| 250-280      | 330-360     | Flavanol (3-OH substituted)                     |
| 250-280      | 350-385     | Flavonol (3-OH free)                            |
| 245-275      | 310-330     | Isoflavone                                      |
|              | 300-330     | Isoflavone (5-deoki-5, 7-dioxygenated)           |
| 275-295      |             | Flavanone and dihidroflavonol                   |
| 230-270 (low power) | 340-390    | Chalcone                                        |
| 230-270 (low power) | 380-430    | Aurone                                          |
| 270-280      | 465-560     | Anthocyanidin and anthocyanin                   |

Quantitative test of flavonoid levels was calculated by using the Dowd method. Two ml 2% aluminum trichloride (AlCl₃) is added to the methanol and mixed to the volume of extract solution (1 mg/ml). The mixture was then incubated at room temperature for 10 minutes, with absorbance 415 nm in spectrophotometer. Total flavonoid was stated in microgram routine. Flavonoid value absorbance can be calculated with formula [19]. The result of quantitative test analysis used 2 ways ANOVA with α=0,05.

$$\text{Absorbance} = 0,0144 \times \text{flavonoid total (μg routine)} + 0,0557$$

$$R^2 = 0,9992$$

3. Result and discussion

3.1. Qualitative flavonoids of antioxidant plants in Pangandaran

Qualitative test was done to investigate the flavonoid of plants assigned. Variation result of positive result of the samples were shown on table 3.

**Table 3. Result of qualitative flavonoid test.**

| No | Extract          | Results          |
|----|------------------|------------------|
| 1  | Leaves of Mahoni | ++++ (Red)       |
| 2  | Leaves of Bungur | + (yellowish)    |
| 3  | Leaves of Vitex  | +++ (orange)     |
| 4  | Leaves of Nyamplung | ++ (yellow-orange_ |

All samples in the study has shown to contain flavonoid in varying level. Flavonoid has been known for decades as the antioxidant found in plants. Many reasearch has reveal the potency of antioxidant found in plant [20,21]. Based on this qualitative studied it was found out that flavonoid in Mahoni leaves was the highest and Bungur showed the least. Qualitative result was then confirmed by flavonoid type test and quantitative test.

Flavonoid type of each plant leaf was determined by UV-Vis spectrophotometer. Absorbance spectrum with the formation of band peak indicated the type of flavonoid on each leaf plant (Table 4).
Table 4. Type of flavonoids of antioxidant plants in Pangandaran.

| Leaves     | Type of Flavonoids              |
|------------|---------------------------------|
| Bungur     | Anthocyanidin dan anthocyanin   |
| Vitex      | Flavanol                        |
| Mahoni     | Isoflavone                      |
| Nyamplung  | Anthocyanidin dan anthocyanin   |

The results of UV - Vis for Bungur leaf and Nyamplung leaves indicated that those leaves contain anthocyanidins and anthocyanins. Bungur showed highest absorbance band in 460 nm only whilst, Nyampung showed two high absorbance bands in 280 nm and 470 nm. The absorbance spectrum of Mahony showed band peak in 260nm and 330nm. Vitex showed sharp band in 280 and 360 nm (Figure 1).

Anthocyanin is natural antioxidant that able to prevent cancer, heart, and high blood pressure diseases. Anthocyanin it is present in all networks of higher plants, including leaf, the branch/trunks, roots, flowers, and fruit. The role of anthocyanin as antioxidant was due to a cluster of hydroxyls on the rings B as the catcher of free radicals, and as antilipo-prooxidants, and degraded collagen caused by radical superoxide anion. The compound also reported could hinder an enzyme that produces radical superoxide, as xanthine [22]. Other study also strongly suggested Nyamplung as antioxidant in plant due to it flavonoid content [23].

Mahoni flavonoid was identified to be categorized as isoflavone. The result was confirmed by previous study which inform flavonoid type of mahoni seed as isoflavone [24]. As mentioned by Markham in Rahmawan [25] the compound was categorized as isoflavone.

The result was supported by study done by Mesaik et al. which found casticin as the flavonoid type found in vitex [26]. Based on identification, casticin was included in the subfamily of flavonol. Casticin, as a flavonoid isolated from Vitex was found to be a potent immunomodulatory and cytotoxic compound. Moreover, casticin compound isolated from many plants has also proven as potential source of antimalaria [27].
3.2. Flavonoid content of some antioxidant plants in Pangandaran

Flavonoids are a type of secondary metabolites. Flavonoids can be found in organs of plants, in this case we use the leaves. Samples that are used were leaves of vitex, bungur, mahogany and nyamplung. The treatment was done by macerating and drying of the leaves. Analysis was conducted on the present of flavonoid, the type and the level. Result of flavonoid content was shown below (Figure 2).

![Figure 2. Levels of flavonoids on Bungur, Mahoni, Nyamplung, and Vitex leaves.](image)

Drying Variations is 14 days and 21 days, where each sample drying followed by maceration for 24 hours and 48 hours. Combined treatment of drying and maceration was studied to acknowledge the effect of processing the leaves on flavonoid content. Quantitative test results maceration and drying effect on levels of flavonoids were tested by 2-way ANOVA. Significant result was showed obtained significant results in all leaves samples with α = 0.05.

The effects of maceration and drying were proven significant to affect the quantity of flavonoid of the leaves. The drying technique used in research was windy drying to avoid direct contact with the sun excessively. The aim of this technique of was to reduce of water content in the leaves so the dominant content of leaves merely secondary metabolites. This technique was supported by research from Sutjipto, et.al that the highest-level results of flavonoid was obtained by the windy-drying techniques [28]. Excessive sun intensity feared will expel the entire component in the leaf tissues.

In all leaves samples results showed that the optimum levels of flavonoids in varying drying. According to the results obtained that variations in the drying and maceration optimum drying is 14 days with maceration of 24 hours in which the results for the bungur leaves level was 37.60 μg routine, mahoni leaves routine is 62.60 μg routine, nyamplung leaves is 37.39 μg routine, and vitex leaves is 56.00 μg routine. In addition, variations in the drying and other drying optimum maceration of 21 days with maceration of 48 hours in which the results for the bungur leaves level was 57.70 μg routine, mahoni leaves is 60.34 μg routine, and vitex leaves is 61.76 μg routine, while the variation drying 21 days with maceration of 48 hours there is a decrease in the yield levels of leaves nyamplung. So, for drying 14 days with maceration 24 hours got optimum results in all types of leaves, while for drying 21 days with maceration 48 hours optimum results are only three types of leaves, because it does not include leaves nyamplung. The result of a sharp decline or can be said to be optimum based on the results is the variation of drying 14 days with maceration of 48 hours in which the results for the concentration of the
bungur leaves is 3.15 μg routine, mahoni leaves is 6.49 μg routine, nyamplung leaves is 3.36 μg routine, and vitex leaves is 4.89 μg routine.

4. Conclusion
It can be concluded that all plants tested positively contain flavonoid with different type. Flavonoid content also varying each other. Immersion as well as drying has also significantly affected the quantity of flavonoid in plants leaf showed by different amount found with each experiment.

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