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Total Flavonoid Content and Antioxidant Activity in Leaves and Stems Extract of Cultivated and Wild Tabat Barito (*Ficus deltoidea* Jack.)

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Abstract. Tabat barito (*Ficus deltoidea* Jack) is a name given by Dayak Tribe who lived in Borneo-Kalimantan and it is belongs to the moraceae. Almost all of the parts of *F. deltoidea* plant is widely used as a medicinal property. The total flavonoid content (TFC) and antioxidant activity from cultivated and wild *F. deltoidea* leaves and stems extract were assessed. Total flavonoid content was estimated by using Aluminium chloride colorimetric method and expressed as catechin equivalents (mg CE g^{-1} extract) and the antioxidant activity by the DPPH (2,2-diphenyl-1-picryl hydrazyl) method. The content of total flavonoid of leaves and stems (430.77 and 371.80 µg CE mg^{-1} extract) of cultivated *F. deltoidea* were higher than in the wild leaves and stems (114.82 and 66.67 µg CE mg^{-1} extract). The IC50 of leaves extract of cultivated and wild *F. deltoidea*, based on the DPPH assay, has a strong antioxidant activity (34.19 and 39.31 µg mL^{-1} extract) as compared to stems extract. These results showed that the cultivated *F. deltoidea* are suitable source for medicinal properties and the leaves could be exploited as source of natural antioxidants.

INTRODUCTION

Tabat barito (*Ficus deltoidea* Jack), a member of moraceae, is an indigenous plant to South East Asia to Borneo, and the Philippines. It is an epiphyte and found in all forest ecosystems except mangrove swamps. According to the Borneo native, tabat barito plant usually used by the women that just birth, regain energy, repair bloods flows and its associated problem, and can be used as herbs drinking for healthy and beauty. Herbal plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils, which is stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc. [1].

Almost all of the parts of *F. deltoidea* are believed to have medicinal properties [2]. Based on the several research *F. deltoidea* having different pharmacological activities of their different parts such as, anti-bacteria [3], anti-microbial [4], anti-aging and anti melanogenic [5], anti-inflammatory [6,7], antioxidant [8], antidiabetic [9], antinociceptive [10,11] and wound healing activities [12]. Aris et al. [13] reported that the methanol extract of fruit *F. deltoidea* has a strong antioxidant activity. The hot water extract from dried *F. deltoidea* leaves has potential as a good source of phenolic antioxidants [14]. A wide range of antioxidants from both natural and synthetic origin has been proposed for use in the treatment of various human diseases [15].

Flavonoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Oxidation is one of the most

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important processes, which produce free radicals in food, chemicals and even in living systems. Free radicals have an important role in the processes of food spoilage, chemical materials degradation and also contribute to more human disorders in human beings. Active oxygen and in particular, free radicals are considered to induce oxidative damage in bio molecules and to play an important role in aging, cardiovascular diseases, cancer and inflammatory diseases [16]. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is to add free radical scavenging assay of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) stable radical. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases [17]. Several researchers suggested the relevance of identifying the metabolic differences between wild and cultivated specimens to assess their quality during the domestication process [18]. In some instances, the wild forms of plants may demonstrate different phytonutrient profiles from the cultivated ones. Several studies have stated that the wild species preserve the different biological capacity and secondary metabolites, compared with the cultivated species [19,20]. According to previous literature, reports illustrating the importance of *F. deltoidea* as a medicinal plant. These plants are available in Borneo. This study aimed to evaluate the total flavonoid content and the antioxidant activity of the methanolic extracts in leaves and stems of cultivated and wild *F. deltoidea*. In addition, the objective of this study was to establish differences between the cultivated and the wild and to evaluate which is the most interesting. The results of this study highlighted the possibility of using *F. deltoidea* organs as a medicinal plant and a potential source of natural antioxidants.

MATERIALS AND METHODS

Cultivation of Ficus deltoidea

Cuttings of *F. deltoidea* were grown in plastic boxes (10 x15 cm) until 160 days before being used in this experiment. Plants were maintained for 12 months before used for testing total flavonoid contents and antioxidant activity. Experiment was done in greenhouse Faculty of Mathematics and Natural Sciences Mulawarman University, Samarinda East Borneo (Samarinda: latitude: 0°21'18" - 1°09'16" (S), longitude: 116°15'36"-117°24'16" (E), altitude: 0.102 km). The wild *F. deltoidea* was collected from Banjarbaru South Kalimantan. (Banjarbaru: latitude: 3°27'26.07" (S), longitude: 114°48'37.14" (E), altitude: 0.306 km

Preparation of plant material

The fresh leaves and stems were washed with tap water and then thoroughly cleaned with distilled water and shade dried for a week. The dried leaves and stems were grinded to a fine powder by using blender. Each leaves and stems powder (150 g) was taken and macerated with 98% methanol. They were kept at room temperature for 6 days. Thereafter the mixtures were filtered using Whatman filter paper no.1. The supernatant were pooled together, concentrated in rotary evaporator at 40°C. The dried extract was used directly to estimate the total flavonoid content and the antioxidant activity.

Determination of total flavonoid contents

The aluminum chloride colorimetric method was used to measure the total flavonoid content of all plant extracts [21]. Extract solution (0.1 mL, 1 mg 10^{-1} mL^{-1}) of each plant extract was added to 0.70 mL of distilled water. Sodium nitrite solution (0.10 mL, 5%) was added to the mixture followed by incubation for 5 minutes after which 0.10 mL of 10% aluminium chloride was added. The mixture was allowed to stand for 6 minutes at room temperature before 0.5 mL of 1 M sodium hydroxide was finally added. The absorbance of the reaction mixture was measured at 510 nm with a UV-VIS spectrophotometer (Shimadzu Corp., Kyoto, Japan) immediately. Catechin was used as the standard for the calibration curve. Flavonoid contents were expressed as µg catechin equivalent (CE) mg^{-1} extract.

Free radical scavenging assay (DPPH)

The DPPH method has been widely used to measure the antioxidant capacities of different residual and natural products. It is a rapid, simple, sensitive and practical assay [22-24]. The free radical 2,2-diphenyl-1-picrylhydrazyl

DPPH scavenging assay was done using the procedure described by Wahid et al. [14], with slight modification. The stock solution of the plant extract was prepared in ethanol to achieve a concentration of 100µg/mL. Dilutions were made to obtain concentrations of 100µg mL^{-1}, 50µg mL^{-1}, 25µg mL^{-1}, 12,5µg mL^{-1} and 6,25µg mL^{-1}. A-33µl mL of each of the diluted solutions was mixed with 467µl absolute ethanol and 0.5mL of DPPH ethanolic solution (27%). After 20 minutes in the darkness at room temperature (23^0C), the absorbance was recorded by a UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 517 nm. The control sample contained all the reagents except the extract. Ascorbic acid was used as standard. The radical scavenging activity of each sample was calculated according to the following formula for inhibition percentage of DPPH:

$$\text{Ip DPPH } (\%) = \left(\frac{\text{Ab} - \text{Aa}}{\text{Ab}}\right) \text{x } 100$$

where, Ip = inhibiton percentage; Aa = the absorbance value of test; Ab = the absorbance value of the blank

Data analysis

The total flavonoid content and antioxidant activity are expressed as the mean±Standard Deviation using Microsoft Excel 2007 and all analyses were done in triplicate. Linear regression analysis was used to calculate IC_{50} value. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine the significance of the difference among samples, with a significance level of 0.05.

RESULTS AND DISCUSSION

Determination of total flavonoid contents

The total flavonoid contents of the leaves and stem of cultivated and wild *Ficus deltoidea* are exhibited in **TABLE 1**. Total flavonoid was expressed in terms of catechin equivalent (CE) per mg of the methanolic extract. The leaves methanolic extract of cultivated and wild *F. deltoidea* contained large amount of total flavonoid (430.77±0.031 and 371.80±0.004 µg CE mg^{-1} extract) when compared to cultivated and wild stems (114.82±0.003 and 66.67±0.003 µg CE mg^{-1} extract). As shown in figure 1 these results indicate that the total flavonoid contents are high in leaves when compared to stem, both in cultivated and wild *F. deltoidea*. This high flavonoid content is responsible for the bioactivity of these crude extracts. Phenolic compounds have redox properties, which allow them to act as antioxidants [25]. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity.

Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity in vitro and also act as antioxidants in vivo [26,27]. The high phenolic and flavonoid content is responsible for the bioactivity of these crude extracts. Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases [28]. Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses [29]. Similarly, phenolics conferring oxidative stress tolerance on plants. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in flavonoids are increasingly being used in the food industry for their antioxidative properties and health benefits.

Estimation of radical scavenging activity by DPPH method

DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up [15].The free radical scavenging activity of *Ficus deltoidea* was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of leaves and stems extracts are shown in **TABLE 2**.

TABEL 1.The Flavonoid content of the methanolic extract from different *F. deltoidea* parts.

Plant Parts	Total Flavonoid Content (µg CE mg^{-1} extract)
Cultivated leaves	430.77±0.031[a]
Wild leaves	371.80±0.004[b]
Cultivated stems	114.82±0.003[c]
Wild stems	66.67±0.003[d]

The values are presented the mean of three replicates ± the standard deviation. The data marked with different letters share significance at $p < 0.05$ (based on the Duncan test).

TABEL 2. The IC_{50} values of leaves and stems of *Ficus deltoidea* of DPPH radical scavenging assay (µg/ml)

Plant parts	IC_{50} (µg mL^{-1} extract)
Cultivated leaves	34.19±0.085[d]
Wild leaves	39.31±0.042[c]
Cultivated stems	205.30±0.029[b]
Wild stems	230.87±0.028[a]
Ascorbic acid	4.14±0.019[e]

IC_{50} = the concentration at which DPPH radicals are scavenged by 50%. The values are presented as the mean ± the standard deviation (n = 3). Mean values with different letters within a column are significantly different ($p < 0.05$).

In DPPH assay, the methanolic leaves extract (both in cultivated and wild *F. deltoidea*) showed more antioxidant activity when compared to methanolic stems extract. It was observed that cultivated and wild leaves extract showed high radical scavenging activity (IC_{50} value = 34.19 and 39.31 µg mL^{-1}) when compared to stems extract that was found to be IC_{50} = 205.30 and 230.87 µg mL^{-1} respectively. IC_{50} value of the standard ascorbic acid was found to be 4.14 µg mL^{-1}. Graphical representations of leaves and stems extracts are shown in **FIG.2**. Antioxidant is one of the most essential ingredients of today's therapy since they reduce *in vivo* oxidative damages. Plants are the good resources for natural antioxidants [30]. Therefore, the great interest has been recently focused on searching of plants having natural antioxidants and good medicinal value. The present investigation revealed that the leaves extract of cultivated and wild *F. deltoidea* contain significant amount of total flavonoids and good antioxidant potential which can be correlated to its medicinal values. This confirms the assertion that flavonoids content of plants contribute directly to their antioxidant properties. The DPPH scavenging capacity of the plant extracts may therefore be related to the flavonoids compounds present.

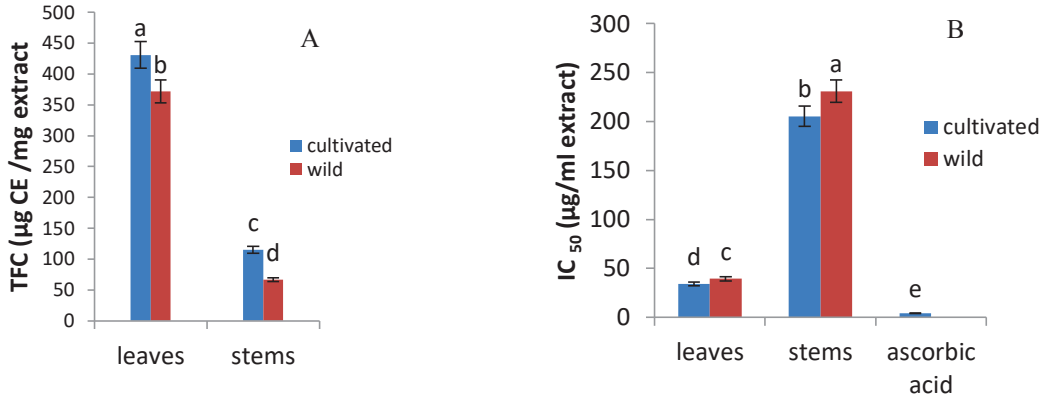

Figure 1. (A) The total flavonoid content of methanolic leaves and stems extract of cultivated and wild *F. deltoidea*; (B) Evaluation of DPPH free-radical scavenging activity of methanolic leaves and stems extract of cultivated and wild *F. deltoidea*

CONCLUSION

Our results indicated that the *Ficus deltoidea* is suitable for cultivation. The cultivated and wild-grown *Ficus deltoidea* organs had different contents of flavonoids and antioxidant activity. The flavonoids content and antioxidant activity of leaves have the highest levels in the cultivated as compared to the stems. These results indicated that the cultivated and wild-grown *F. deltoidea* possess strong antioxidant activities with significant differences. Therefore, the leaves of this plant could be a prospective source of natural bioactive molecules that could replace synthetic antioxidants.

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