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Total flavonoid content and antioxidant activity of tabat Barito (*Ficus deltoidea* Jack) on different plant organs and ages

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Abstract
The greenhouse study was conducted to determine the phytochemical compound, total flavonoid content (TFC), and antioxidant activity on different plant organs and ages. The tests were performed on fresh leaves, senescent leaves, unripe fruit, ripened fruit, and stem at 6, 9, and 12 months old after planting (MAP). The results showed that the phytochemical compound different indifference organs and ages. The older age of the plant, the more types of the phytochemical compound found within each plant organ. At 6 and 9 MAP, the highest flavonoid content was found in senescent leaves extract and the lowest found on the stem. Whereas at the 12 MAP, the highest TFC was found on senescent leaves extract followed by ripened fruit, fresh leaves, unripe fruit, and stem. Antioxidant activity in all organs studied increased with increasing age of the plant. Based on IC_{50} values, at 12 MAP, the highest antioxidant activity was obtained on senescent leaves, followed by fresh leaves, ripened fruit, unripe fruit, and the lowest on the stem. The result of the present study showed that the senescent leaves extract has the highest total flavonoid content and antioxidant activity at 12 MAP as compared to fresh leaves, unripe fruits, ripened fruits, and stem.

Keywords: *Ficus deltoidea*, flavonoid, different organ, fresh, senescence, ripe, unripe

1. Introduction
Tabat barito (*Ficus deltoidea* Jack) is a native and widely distributed in South Asia (Fatihah *et al*., 2014) [1] include Indonesia, Thailand, and Malaysia (Lansky & Paavilainen 2011) [2]. It is a small shrub and epiphytic (Brickell and Zuk, 1997) [3]. It can be easily found along shrubland beaches and hilly forest, but do not found in the mangroves (De Padua *et al*., 1999) [4]. Tabat barito is one of the most medicinal plants in Kalimantan-Indonesia. In recent year, the popularity of medicinal plants is increasing, as more and more people are using herbal medicine in their daily life. In Indonesia, especially in Kalimantan, *F. deltoidea* is one of the medicinal plants that widely used by local communities. This plant is very valuable and very expensive in traditional markets because it is widely used as a traditional medicine (Sulaiman *et al*., 2008) [5]. This is because the presence of various types of phytochemical compounds makes these plants a valuable source of medicinal (Anwar *et al*., 2007) [6]
Based on a recent study, *F. deltoidea* proven to have biological activity such as: antinociceptive (Ahmat *et al*., 2008) [7], anti-inflammatory (Abdullah *et al*., 2009) [8], (Zakaria *et al*., 2012) [9], antidiabetes (Adam *et al*., 2012) [10], (Misbah *et al*., 2013) [11], antiobesity (Woon *et al*., 2014) [12], antimelanogenic (Oh *et al*., 2011) [13], antioxidants (Hakiman *et al*., 2012) [14], (Misbah *et al*., 2013) [11], free radical fishing activity (Norra, 2011) [15]. Hakiman *et al*. (2012) [14] and Misbah *et al*. (2013) [11] reported that leaf extract and fruit extract of *F. deltoidea* have phenolic and flavonoids compounds. Phytochemical compound that contained in *F. deltoidea* such as phenol and flavonoids make this plant has a high biological activity. Flavonoids are a group of phenolic compounds that have high antioxidant activity. Gryglewski *et al*. (1987) [16] reported that the high biological activities of phenol and flavonoids related to their antioxidant activity.
The community needs for *F. deltoidea* especially in Kalimantan is increasing, so this plant has started to be cultivated. Almost all parts of these plant are believed to have medicinal properties (Adam *et al*., 2007) [17]. Although *F. deltoidea* is an epiphytic plant, this plant can be planted on the ground (Musa, 2006) [18]. The growth of *F. deltoidea* is better in soil media as compared to sand media (Arifin *et al*., 2011) [19]. The nitrogen treatment on the cultivation

F. deltoidea. increased the growth and the antioxidant activity of leaf (Sheikh and Ishak, 2016) [20]. In plants that undergoing cultivation process, the phytochemical compound that has an economic value such as phenolics and flavonoids must be preserved, so they can still be used as medicines (Donno *et al*., 2013) [21]. The phytochemical, flavonoids, differ in each species, ages, and parts of plants (Baikar and Malpathak, 2010) [22].

However, despite the fact that *F. deltoidea* is interesting from a medicinal, pharmacological, and agronomical point of view, the flavonoid content and the antioxidant activity in different organs and different ages unevaluated. Therefore, the purpose of this study was to evaluate the total flavonoid content and the antioxidant activity of tabat barito (*Ficus deltoidea* Jack) on different plant organs and ages.

Materials and Methods

Soil and plant selection

Preparation Soil and Plant Material

The topsoil was analyzed before use as a planting medium of *F. deltoidea* (pH = 6.12, N = 0.688%, P = 21 ppm, K = 0.66 cmol(+)/Kg, Ca = 4.84 cmol(+)/Kg, Mg = 4.41 cmol(+)/ Kg), then added NPK Mutiara (16:16:16) as a basic fertilizer. Tabat barito seedlings from 16-week-old stem cuttings are used as plant material. The study was conducted in the glass house of FMIPA UNMUL Samarinda (coordinates: 0°21'18" - 1°09'16" South and 116°15'36" - 117°24'16" East). Seedlings planted in planting pots (diameter 35 cm, height 25 cm) that contain 15 kg of soil. Watering is done once per 2 days, and the plants are maintained until one-year-old (12 months). Plant part organs (fresh leaves, senescent leaves, unripe fruit, ripened fruit, and stems) were taken after 6, 9, 12 month after planting (MAP) and testing for the phytochemical profile, total flavonoid content, and antioxidant activity.

Extraction of plant materials

The fresh leaves (FL), senescent leaves (SL), unripe fruit (UF), ripened fruit (RF), and stems (ST) (Figure 1.) are separated and dried indoors for 7 days at 20°C, then ground using a blender. Ground FL, SL, UF, RF, and ST were extracted with 98% methanol, placed on the shaker for 3 days, then filtered using whatmann no filter 2. This process was repeated for 3 times. After filtration, the crude methanol extract was evaporated using a rotary evaporator at 40°C and placed in a vacuum oven to near dryness the extract. The yield of methanol extract was used for testing the phytochemical profile, the total flavonoid content and the antioxidant activity.

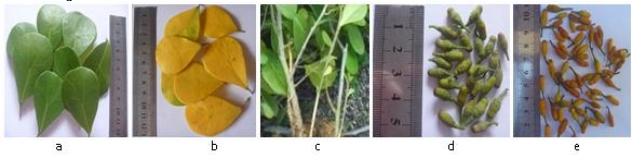

Fig 1: a. fresh leaves (FL), b. senescent leaves (SL), c. stems (ST), d. unripe fruits (UF), and e. ripened fruits (RF) of *f. deltoidea*.

Phytochemical Screening

One gram of each dried methanol extracts (FL, SL, UF, RF, ST) was dissolved in 100 mL methanol and subjected to preliminary phytochemical screening following standard methods (Harborne, 1998) [23], (Kokate, 2001) [24]. The phytochemical test was performed to detect the presence of phytochemicals in the extract, they are: (1)Test for Alkaloid-Dragendroff's test: A few mL extract solution (2 mL) added with 3-4 drops dragendroff's reagent. The changes color of the solution into red or orange indicates that the extract contains alkaloids. [2] Test for Flavonoid: A-2 mL crude extract solution dissolved in 5 mL of water, boiled for 5 minutes and then filtered. Two mL filtrate was added with 0.05 mg of Mg powder and 1 mL chloride acid, then shaken until homogeneous. The formation of yellow or red color indicates the presence of flavonoids [3]. Test for Phenol, To 2 mL of extracts solution added with 3-4 drops of ferric chloride solution. The formation of bluish-black colour indicates the presence of phenols [4]. Test for Saponin, to a few mL crude extract solution (2 mL) added with 5 mL of distilled hot water and then shaked vigorously until 10 minutes. The formation of a layer of foam indicated the presence of saponins [5]. Test for Steroid and [6] Triterpenoid, Liebermann-Burchard's test: 2-mL Extract was added with 1 mL chloroform and a few drops of acetic anhydride. Concentrated H_2SO_4 was added slowly through the sides of the test tube to form a layer. The formation of blue-green colour indicates the presence of steroids, the formation of a reddish brown color indicated the presence of terpenoids [7]. Test for Tannin, to a 0.5 g crude extract added 10 mL distilled water, boiled and then filtered. About 2-3 drops of 0.1% ferric chloride were added to the filtrate. Formation of green or blue-black colour indicates the presence of tannin [3]. Test for Coumarin, to one mL crude extract solution was added with 3-4 drops of NaOH and dissolved with 2 mL alcohol. The changes of extract solution to yellow colour indicate that the extract contains coumarins [9]. Test for Carotenoid, One mL extract dissolved with 5 mL chloroform in a test tube, shaked, and 85% sulfuric acid was added slowly. The formation of blue color in the upper layer indicated the presence of carotenoid.

Determination of Total Flavonoid Content (TFC)

The total flavonoid content of each plant extract was determined by using Aluminum chloride colorimetric technique (ACCT) method (Kumari & Sharma, 2015) [25]. One mg of methanolic extract dissolved with 10 mL DMSO and used an extract solution. 5% NaNO2 solution (5 mg in 100 ml of distilled water), 1M NaOH solution (4 mg in 100 ml of distilled water), and 10% AlCl3 solution (10 mg in 100 ml of methanol) was prepared. The test was performed on 0.1 ml of extract solution added with 0.7 ml of distilled water, 0.1 ml NaNO2 5%, 0.1 ml AlCl3 10% and 0.5 ml 1M NaOH and then incubated for 10 minutes in a dark room. The absorbance was measured at 510 nm using UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan) against a blank. The standard curve was prepared using catechin by dissolving it in DMSO followed by serial dilution to 2, 4, 6, 8, 10 µg/ml. Total content of flavonoid in the plant extracts were expressed as µg catechin equivalents (CE)/mg extract and were calculated by the formula: $T = (C \times V)/M$

Where, T = total content of phenolic compounds (µg of CE mg^{-1} extract)

C = the concentration of catechin established from the calibration curve (µg mL^{-1})

V = volume of extract (mL)

M = weight of methanolic plant extract (mg)

Determination of Antioxidant Activity

The antioxidant activity was measured employing the modified method of Arung *et al*. (2009) [26]. The stock solution of each FL, SL, UF, RF, and ST extract were prepared by dissolved 3 mg dried extract with 1 mL ethanol. Dilution was made to obtain the concentration of 100, 50, 25,

12.5, and 6.25 ppm. Diluted solution (33 µL each) was added to 467 µL of ethanol and 500 µL of 27% DPPH solution. The mixed solution was incubated for 20 minutes in the darkness, then the absorbance was measured using a UV-VIS spectrophotometer at 517 nm. Vitamin C was used as a standard. Percentage of antioxidant activity was calculated using the equation:

$$\% \text{ Antioxidant activity} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100\%$$

The IC_{50} value was calculated using linear regression of plots where the abscissa represented the concentration of extract solution and the ordinate was the percent of the antioxidant activity. The lower IC_{50} value indicated the higher antioxidant activity.

Statistical analysis

The total flavonoid content and antioxidant activity are expressed as means ± standard error (SE), the IC_{50} value was calculated using linear regression analysis. The data on TFC were analyzed using SPSS version 22 (SPSS, Inc., USA). The data were compared using one-way ANOVA, followed by Duncan post hoc test to evaluate significant differences among plant organs and ages with a confidence level 0.05.

Results and Discussions

Phytochemical Screening

The methanolic extracts of FL, SL, UF, RF, and ST were screened for the presence of alkaloid, tannin, phenolic, flavonoid, steroid-triterpenoid, coumarin, carotenoid, and saponin on 6 MAP (Table 1), 9 MAP (Table 2) and 12 MAP (Table 3). The results showed that phytochemical compound differs in different plants organs and ages. The alkaloids were presence for the FL, SL, ST extract, absence for the UF and RF extract at 6 MAP, but their presence for all the plant part organs extract at 9 and 12 MAP. At 6 MAP, phenolic and flavonoid only detected on SL, UF, RF extracts, whereas at 9 and 12 MAP phenolic and flavonoid detected in all plant parts extract. Tannin was detected only in ST extract on all plant ages. Coumarin undetected in all plant parts organ on 6 MAP; it was detected in UF, RF and ST extract on 9 MAP. The FL, SL, UF, RF, and ST extract tested negative for the presence of saponin on all plant ages. All plant part organ extract showed the absence of saponin. The presence of phytochemicals in the fresh leaves, senescent leaves, unripe fruits, ripened fruits, and stems extract may be due to variations of phytochemical distribution in the different plant parts. Distribution of phytochemicals such as phenol, alkaloid, and flavonoid varies on different plant parts and different plants species in the vascular plant (Harborne, 1998) [23]. The phytochemical compound detection in plants is dependent on factors such as age, climate, plant part, habitat, season, time of harvest, chemical races of plants and sensitivity of phytochemical (Nduagu *et al*., 2008) [27]. Our recent study showed that the older age of the plant more and more types of phytochemicals detected. Alkaloids have been reported as an anti-inflammatory (Souto *et al*., 2011) [28], antimicrobial (Benbott *et al*., 2012) [29], and antimalarial (Dua *et al*., 2013) [30]. Steroids are known to have a cardiotonic effect and also possess antibacterial and insecticidal properties (Bagrov *et al*., 2009) [31]. Tannins are known to have antibacterial (Akiyama *et al*., 2001) [32], antitumor and antiviral activities (Kumari and Jain, 2012) [33], Saponin has hypotensive and cardio depressant (Olaleye, 2007) [34]. Flavonoids and tannins are phenolic resin compounds that may act as primary antioxidants or free radical scavengers. In medicinal plants, the phytochemical compound like alkaloids, saponin, tannins, and flavonoids have antipathogens and antimicrobial activities (Ghosh *et al*., 2010) [35], coumarin has an anticoagulant activity (Weigt *et al*., 2012) [36].

Table 1: Phytochemical screening of different plant parts of *F. deltoidea* on 6 MAP

Phytochemical test	Fresh leaves	Senescent leaves	Unripe fruits	Ripened fruits	Stems
Alkaloid	+	+	-	-	+
Tannin	-	-	-	-	+
Phenolic	-	+	+	+	-
Flavonoid	-	+	+	+	-
Steroid	+	+	+	+	+
Kumarin	-	-	-	-	-
Karotenoid	-	-	-	-	-
Saponin	-	-	-	-	-

Remarks: *Plus sign indicates presence and minus sign indicates absence.

Table 2: Phytochemical screening of different plant parts of *F. deltoidea* on 9 MAP

Phytochemical test	Fresh leaves	Senescent leaves	Unripe fruits	Ripened fruits	Stems
Alkaloid	+	+	+	+	+
Tannin	-	-	-	-	+
Phenolic	+	+	+	+	+
Flavonoid	+	+	+	+	+
Steroid	+	+	+	+	+
Kumarin	-	-	+	+	+
Karotenoid	-	-	-	-	+
Saponin	-	-	-	-	-

Remarks: *Plus sign indicates presence and minus sign indicates absence.

Table 3: Phytochemical screening of different plant parts of *F. deltoidea* on 12 MAP

Phytochemical test	Fresh leaves	Senescent leaves	Unripe fruits	Ripened fruits	Stems
Alkaloid	+	+	+	+	+
Tannin	-	-	-	-	+
Phenolic	+	+	+	+	+
Flavonoid	+	+	+	+	+
Steroid	+	+	+	+	+
Kumarin	+	+	+	+	+
Karotenoid	-	-	-	-	+
Saponin	-	-	-	-	-

Remarks: *Plus sign indicates presence and minus sign indicates absence.

Total Flavonoid Content (TFC)

The total flavonoid content of fresh leaves, senescent leaves, unripe fruit, ripened fruit, and stems on different plant ages are exhibited in Table 4 and there were expressed in term of catechin equivalent (CE) per mg of dried methanolic extract. From the result obtained, at 6 and 9 MAP, the highest total flavonoid content was found in the RF extract as compared to UF, SL, FL, and ST extracts. Whereas at 12 MAP the highest TFC was obtained in SL extract when compared to others plant parts extract. The ripened fruits extract has the highest TFC as compared to unripe fruits; the senescent leaves extract has the highest TFC as compared to fresh leaves. As plant ages increased from 6 to 9 MAP, the total flavonoid content of FL, SL, UF, RF, and ST were increased about 52.07%, 41.00%, 1.71%, 11.81% respectively. When plant ages increased from 9 to 12 MAP, the TFC of FL, SL, UF, and RF

were increased about 22.45%, 28.95%, 4.14%, 2.39%, whereas ST decreased about 11.68%. Our results indicated that the increasing age of *F. deltoidea* total flavonoid content of all plant organs studied was improved except the stems. At 12 MAP, the total flavonoid content was highest in senescent leaves extract followed by ripened fruits, fresh leaves, unripe fruits, and stems. This is the first report of the total flavonoid content of various plant organs of *F. deltoidea* on different plant ages. Our results are in line with Cirak *et al*. (2014) [37], who reported that the flavonoid quantities of *Hypericum pruinatum* different among plant organs depending on the developmental stage and reproductive parts, and the leaves accumulated the highest level of flavonoids. Kumari & Sharma, (2015) [25] also reported that the flavonoid content of *Oxalis corniculata* is high in leaves when compared to the stem.

Table 4: Total flavonoid content of different plants organs and ages of F. *deltoidea*

Plant organs extract	Total flavonoid content (µg CE/mg extract)		
	6 MAP	9 MAP	12 MAP
Fresh leaves (FL)	206.67 ± 0.023^{d3}	314.29 ± 0.055^{d2}	369.05 ± 0.052^{c1}
Senescent leaves (SL)	256.67 ± 0.040^{c3}	361.91 ± 0.044^{b2}	466.67 ± 0.036^{a1}
Unripe fruits (UF)	328.21 ± 0.037^{b3}	333.81 ± 0.026^{c2}	347.62 ± 0.047^{d1}
Ripened fruits (RF)	374.36 ± 0.020^{a3}	418.57 ± 0.031^{a2}	428.57 ± 0.037^{b1}
Stems (ST)	138.67 ± 0.029^{e3}	173.33 ± 0.032^{e1}	153.09 ± 0.026^{e2}

The different letter indexes (a, b, c, d) in the same colum indicate significantly different means for different plant organs and the different numerical indexes (1, 2, 3) in the same row indicate significantly different means at different plant ages at $P < 0.05$.

Antioxidant Activity

The antioxidant activity of different plant organs and ages of *F. deltoidea* was evaluated by DPPH method. The results of the antioxidant activity based on the IC_{50} values are presented in Table 5. At 6 MAP, the senescent leaves extract showed higher antioxidant activity (IC_{50} = 57.257 ± 0.029 µg mL^{-1}) followed by ripened fruits (IC_{50} = 71.068 ± 0.043 µg mL^{-1}), fresh leaves (IC_{50} = 78.099 ± 0.015 µg mL^{-1}), unripe fruits (IC_{50} = 97.451 ± 0.022 µg mL^{-1}) and stems (IC_{50} = 287.946 ± 0.045 µg mL^{-1}). IC_{50} value of the standard ascorbic acid was found to be 5.33 µg mL^{-1}. At 9 MAP, senescent leaves extract showed higher antioxidant activity (IC_{50} = 51.906±0.034 µg mL^{-1}) followed by ripened fruits (IC_{50} = 53.112 ± 0.071 µg mL^{-1}), fresh leaves (IC_{50} = 59.492 ± 0.031 µg mL^{-1}, unripe fruits (IC_{50} = 85.088 ± 0.030 µg mL^{-1}) and stems (IC_{50} = 135.062 ± 0.064 µg mL^{-1}). Whereas at 12 MAP, the senescent leaves extract showed higher antioxidant activity (IC_{50} = 34.190 ± 0.003 µg mL^{-1}) followed by fresh leaves (IC_{50} = 34.473 ± 0.037 µg mL^{-1}), ripened fruits (IC_{50} = 39.457 ± 0.068 µg mL^{-1}), unripe fruits (IC_{50} = 97.451 ± 0.022 µg mL^{-1}) and stems (IC_{50} = 126.137 ± 0.037 µg mL^{-1}).

Our results revealed that the senescent leaves extract has the higher antioxidant activity as compared to fresh leaves, unripe fruits, ripened fruits, and stems; the older age of *F. deltoidea* the higher the antioxidant activity. The trend of increase in antioxidant activity was parallel with the increasing age of *F. deltoidea* up to 12 months old. This activity due to the total flavonoids content that was evaluated in all plant organs. It was observed that the senescent leaves extract at 12 MAP had the higher total flavonoid content, hence the antioxidant activity was also higher at that ages. The study by Ahmed *et al*. (2014) [38] found that the total flavonoid had a positive correlation with the antioxidant activity in *Calamus tenuis* Roxb.

Base on the IC_{50} value, the strength of the antioxidant activity categorized as strong (IC_{50} < 50 ppm), active (IC_{50} = 50-100 ppm), moderate (IC_{50} = 101-150 ppm), and weak (IC_{50} = 151-200 ppm) (Molyneaux, 2004) [39]. The recent study showed that the fresh leaves, senescent leaves, ripened fruits had a strong antioxidant activity; unripe fruits had an active antioxidant activity; stems had a weak antioxidant activity at 12 MAP.

Table 5: The IC_{50} values of different plants organs on different plants ages of *F. deltoidea* of DPPH radical scavenging assay (ppm)

Plant parts extract	The IC_{50} values (ppm)		
	6 MAP	9 MAP	12 MAP
Fresh leaves (FL)	78.099±0.015	59.492±0.031	34.473±0.037
Senescent leaves (SL)	57.257±0.029	51.906±0.034	34.190±0.003
Unripe fruits (UF)	97.451±0.022	85.088±0.030	50.213±0.037
Ripened fruits (RF)	71.068±0.043	53.112±0.071	39.457±0.068
Stems (ST)	287.946±0.045	135.062±0.064	126.137±0.037

Conclusions

The results showed that different organs on different ages have different total flavonoid content and antioxidant activity. The fresh leaves, senescent leaves, unripe fruits, ripened fruits had the higher total flavonoid content at 12-month-old, while the stems were higher at 9-month-old. The older age of tabat barito (*F. deltoidea*) the higher the content of flavonoids in leaves and fruit. The antioxidant activity of all plant organ of *F. deltoidea* was higher at 12-month-old as compared to 9 and 6-month-old. The senescent leave has the higher total flavonoid content and the stronger antioxidant activity as compared to fresh leaves, unripe fruits, ripened fruits, and stems. To our knowledge, this is the first report documenting the detailed changes in total flavonoid content and antioxidant activity in different organs of *F. deltoidea* during its growth cycle.

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