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Growth, phytochemical profile, and antioxidant activity of cultivated tabat barito (*Ficus deltoidea* Jack) under drought stress

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Abstract

Tabat barito (*Ficus deltoidea* Jack.) is one of the most important medicinal plants in Kalimantan. The growth, phytochemical profile, and antioxidant activity were assessed in *Ficus deltoidea* growing under four different water field capacity (FC) condition, namely: W1 (40% FC), W2 (60% FC), W3 (80% FC), and W4 (100% FC-control), and investigated for 9 months. This finding showed that the drought stress treatment reduced the plant height, leaf number, leaf area, leaf thickness, and number of branches, chlorophyll number, and the biomass. The highest value of growth parameters was observed under 100% FC-control. The leaves extract contained four secondary metabolites compounds (alkaloid, flavonoid, phenolic, and steroid), the stems extract contained seven secondary metabolites compounds (alkaloid, flavonoid, phenolic, tannin, steroid, coumarin, and carotenoids), and the fruits have five secondary metabolite compounds (alkaloid, flavonoid, phenolic, steroid, and coumarin) in all drought stress treatment. The highest TPC on the stem extract (74.07±0.001 µg GAE/mg extract), TFC (397.44±0.007 µg CE/mg extract) and antioxidant activity on the leaves extract (IC_{50} = 72.47±0.050 µg/mL) were obtained on W1 40% FC, while the lowest TPC (3.70±0.001 µg GAE/mg extract) and antioxidant activity (IC_{50} = 1238.06±0.003 µg/mL) on the fruits extracts, TFC (146.00±0.001 µg CE/mg extract) on the stem extract were obtained on W4 100% FC. In conclusion, the drought stress treatment (40% FC) was introduced to obtain appreciable the highest TPC, TFC and the antioxidant activity of cultivated *F. deltoidea*.

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Introduction

One of the environmental abiotic factors affecting plant growth and production is drought stress. Generally, drought stress reduced the growth and production of several plants that distributed within the world. The response of plants to water stress is determined by the level of stress experienced and the growth phase of the plant during the stress. Drought stress results in cellular and molecular level change such as decreased in plant growth, cell volume, leaf area, biomass, photosynthesis rates; increased in leaves thickness, leaves protein content, shoot-root ratios, stomatal sensitivity; and changes in nitrogen metabolism, enzyme production, and hormone activity (Geerts and Raes, 2009). Drought stress also affects water content in plants, water turgor pressure, stomatal closure, evaporated and transpiration reduction, and decreased photosynthesis rate (Zhu *et al.*, 2009). Furthermore, drought stress significantly affects the essential oil content, composition, and gene expression of *Origanum vulgare* (Morshedloo *et al.*, 2017).

Recently, medicinal plants have been widely cultivated to provide the needs in the field of pharmaceutical industry and natural cosmetic products. The cultivation of medicinal plants carried out various ways to increase the content of secondary metabolites such as drought stress. Medicinal plants are very beneficial for the people because they contain secondary metabolites that use as medicines.

In plants, including medicinal plants, the production of secondary metabolite compounds strongly influenced by the various environmental factor such as light regime, temperature, the drought stress, etc (Gholizadeh *et al.*, 2010; Das and Bhattacharya, 2016). The study by Gharibi *et al.* (2016) showed that the drought stress increased the total phenolic, total flavonoid, prolin and antioxidant activity of three *Achillea* species (*Achillea millefolium*, *A. nobilis*, and *A. filipendulina*). The amount of secondary metabolite in root bark of *Rhus tripartitum* and *Periploca laevigata* increased significantly under water deficit (Ncib *et al.*, 2018).

Khorasaninejad *et al.* (2011) reported that drought stress significantly decreases all growth parameters; shoot fresh weight, shoot dry weight, root dry weight, internodes length, shoot to root ratio, biomass, and secondary metabolite content; essential oil yield in peppermint (*Mentha piperita* L.). According to Baloğlu *et al.* (2012), the antioxidant enzymes activities in two sunflowers (*Helianthus annuus*) cultivar Aydin and Musala increased as a response to drought stress. Besides enhancing the antioxidative system as a defended to drought stress, the plant also produces the varied secondary metabolite compounds. The production of secondary metabolite compounds such as phenolic and flavonoid is the primary responsibility of the plant to the drought stress. The number of secondary metabolite compounds in the plant namely *Rehmannia glutinosa* (Chung *et al.*, 2006) and pepper (*Capsicum annuum*) (Doaa and Nour, 2012) increased under drought stress. In addition, Nacif and Mazzafera (2005) and Zhu *et al.* (2009) also reported that the drought stress decreased the growth of the medicinal plants *Hypericum brasiliense* Choisy and *Bupleurum chinense*. Though some report of the drought stress has been reported in some plants, the effects of drought stress on the tabat barito (*Ficus deltoidea* Jack.) is little unknown.

Ficus deltoidea is one of the most important plants that used as a medicinal plant in Kalimantan-Indonesia. *Ficus deltoidea*, belongs to the Moraceae family, is an epiphyte and found in all forest ecosystems except mangrove swamps. The tabat barito, a name given by local people in Kalimantan especially Dayak tribe, is widely used as a traditional medicine and very popular in the local community and market as a tea bag. The leaves, fruit, stems, and roots of the *F. deltoidea* are favorite parts that can be used as a medicine, for example, a decoction of the dried leaves is used as a tonic afterbirth. The previous study by Misbah *et al.* (2013) evaluated that the aqueous extract and fraction of fruits *F. deltoidea* has antidiabetic and antioxidant properties. Manurung *et al.* (2017[a]) reported that the methanolic extract of fresh and senescent leaves of *F. deltoidea* has the high

antioxidant activities. The standardized Aqueous ethanolic extract of *F. deltoidea* has an anti-hypertensive (Azis *et al.*, 2017). The antinociceptive activity of leaves aqueous extract *F. deltoidea* has also been documented by Sulaiman *et al.* (2008). Past published work by Hakiman and Maziah (2009) indicated that the fresh leaves aqueous extract of *F. deltoidea* has non-enzymatic and enzymatic antioxidant activities.

In Kalimantan-Indonesia, the utilization of *F. deltoidea* as a medicinal plant is greatly improved. It is harvested directly from the forest and has the potential extinction due to over-harvesting. Thus, this plant is very important to be cultivated without changing its usefulness as a medicinal plant. The cultivation of *F. deltoidea* has been started but to date, there is no report documented on growth analysis, phytochemical screening, total phenolic content, total flavonoid content, and antioxidant activity of cultivated tabat barito under drought stress. Hence, the aim of this research was to investigate the growth, phytochemical profile (total phenolic and total flavonoid content) and antioxidant activity of *F.deltoidea* that cultivated under drought stress.

Materials and methods

Cultivation of tabat barito (*Ficus deltoidea* Jack.)

Ficus deltoidea plants were initiated from cuttings. Cuttings were collected from the mother trees in Banjarbaru, South Kalimantan, Indonesia (Coordinates: 3°22'55"-3°36'22" South and 114°40'35"-114°54'51" East). Cuttings were grown in plastic boxes (10 × 15 cm) filled with topsoil until 160 days before being used as a seedling in this experiment. *F. deltoidea* cultivated in the glass house, Laboratory of plant physiology and development, Biology Department, Faculty of Mathematics and Natural Sciences, Mulawarman University, East Kalimantan, Indonesia (Coordinates: 0°21'18"-1°09'16" South and 116°15'36"-117°24'16" East). Plastic pot (35 x 25 cm) filled with topsoil before seedling transferred and planted into a pot and maintained for 9 months. Four water field capacity (FC) levels were used as treatments of drought stress viz: W1 (40% FC), W2 (60% FC), W3 (80% FC), and W4 (100% FC). The field soil samples were irrigated until saturated and after 24 hours, irrigation soil samples were weighed by using an electrical scale and dried in an electric oven at 105°C for 48 hours. The rate of 100% field capacity was measured following the equation.

$$\theta W = \frac{\text{Moist soil weight} - \text{Dry soil weight}}{\text{Dry soil weight}}$$

The field soil samples were selected to determine soil moisture rate once in a day, then determined 80% FC, 60% FC, and 40% FC respectively (Farahani *et al.*, 2009). The drought stress treatments were applied after first irrigation until plant maturity. The plants were maintained for 9-months (fruiting stage) before harvested. All the plants were harvested for growth evaluation (plant height, leaf number, leaf area, leaf thickness, stem diameter, the number of branches, chlorophyll number, and biomass), phytochemical screening, total phenolic, total flavonoid content, and antioxidant activity.

Preparation of plant material

The harvested-fresh leaves, stems, and fruits of *F. deltoidea* were washed with clean water, dried in a shaded room at 20°C for two weeks. The dried leaves, stems, and fruits were cut into pieces and grinded using a blender. The powder (150 g) was taken and macerated with absolute methanol. They were kept and shaked for 6 days and filtered by using whatmann filter paper. The supernatant was pooled together, concentrated in a rotary evaporator at 40°C. The dried extract was used to screening the phytochemical and calculates the total phenolic content, total flavonoid content, and antioxidant activity.

Phytochemical screening

One gram of each dried methanol extract of leaves, stems, and fruits were dissolved in 100 mL methanol and used to estimate the phytochemical screening by standard methods (Harborne, 1998; Kokate, 2001). The phytochemical test was performed to detect the

presence of phytochemicals in the extract, (1) Test for alkaloid-Dragendroff's test: The Dragendorff"s reagent (3-4 drops) added to 2 mL extract solution. The changes color of the solution into red or orange indicates that the extract contains alkaloids. (2) Test for flavonoid: The crude extract solution (2 mL) dissolved using 5 mL of water, boiled for 5 minutes and then filtered. A-0.05 mg of Mg powder and 1 mL HCl added to 2 mL filtrate, then shaken until homogeneous.

The presence of flavonoids indicated by the formation of yellow or red color in the solution. (3) Test for phenolic: Two mL of extract solution added with 3-4 drops of ferric chloride solution. The formation of a bluish-black color solution indicates the presence of phenols. (4) Test for saponin: A-2 mL extract solution (2 mL) was dissolved with 5 mL of distilled hot water and shaken for 10 minutes. The formation of layer foam indicates positive for saponins. (5) Test for Terpenoid, Liebermann-Burchard's test: Extract was treated with a few drops of acetic anhydride, boiled and cooled. Concentrated H_2SO_4 was then added slowly through the sides of the test tube to form a layer. The presence of terpenoids was indicated by the formation of a reddish brown color. (6) Test for tannin, 0.5 g crude extract was added 10 mL of distilled water, boiled and filtered. Then the filtrate added with 2-3 drops of 0.1% ferric chloride. The color of filtrate turned to green or blue-black color indicate positive of tannin. (7) Test for coumarin: The crude extract solution (1 mL) was added with 3-4 drops of NaOH dissolved with 2 mL alcohol. The changes of the extract solution to yellow color indicate that the extract contains coumarins. (8) Test for a carotenoid: One mL extract mixed with 5 mL chloroform in a test tube, shaken, and 85% sulfuric acid was added slowly. The formation of blue color in the upper layer indicated the presence of carotenoid.

Determination of total phenolic content (TPC)

The total phenolic content of leaves, stems, and fruits of methanolic extracts of *F. deltoidea* were determined spectrophotometrically using Folin-Ciocalteu reagent (Arung *et al.*, 2009) with slight modifications. A-1 mg methanolic extract was dissolved in 10 mL DMSO and used as an extract sample. As a standard solution of gallic acid, one mg of gallic acid was dissolved in 10 mL DMSO. Folin-ciocalteu reagents: to one mL Folin-ciocalteu was added with 9 mL sodium carbonate 7.5% (7.5 mg of sodium carbonate dissolved in 100 mL of distilled water). To 1 mL sample was added with 0.4 mL of distilled water, 0.25 mL Folin-ciocalteau reagents, and 1.25 mL of sodium carbonate. The solution was incubated for one hour in the dark room. Prior to sample testing, a standard calibration curve of the gallic acid solution was prepared (0, 20, 40, 60, 80, 100 µg mL^{-1}). The absorbance was measured at 760 nm using UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The absorbance was measured to determine the total phenolic contents. All determinations were performed in triplicate. The result of the absorbance of the sample incorporated into the linear regression curve of the standard gallic acid to determine the total phenolic content (Kumari and Sharma, 2015). The total phenolic content of leaves, stems, and fruits extracts were expressed as gallic acid equivalents (µg of GAE mg^{-1} sample) and calculated by the formula:

$$T = (C \times V)/M$$

Where, T = The total phenolic content (µg of GAE mg^{-1} sample)

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

V = volume of extract (mL)

M = weight of methanolic plant extract (mg).

Determination of total flavonoid content (TFC)

The total flavonoid content of plant extract was determined by using Aluminum chloride colorimetric technique (ACCT) method (Kumari and Sharma, 2015). One mg of methanolic extract was dissolved in 10 mL DMSO and used an extract solution. 5% $NaNO_2$ solution (5 mg in 100 mL of distilled water), 1M NaOH solution (4 mg in 100 mL of distilled water), and 10% $AlCl_3$ 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 $NaNO_2$ 5%, 0.1 mL $AlCl_3$ 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 in DMSO followed by serial dilution to 2, 4, 6, 8, 10 μgmL^{-1}.

The total content of flavonoid in the plant extracts was expressed as µg catechin equivalents (CE)/mg extract and was calculated by the formula:

$$T = (C \times V)/M$$

Where, T = total content of flavonoid 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). The stock solution of the extract was 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 a 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

Results were expressed as means ± standard error (SE) and data were analyzed using SPSS version 22 (SPSS, Inc., USA). The data were compared using one-way ANOVA, followed by Duncan post hoc with a confidence level of 0.05.

Results

The growth parameters

Data on the growth parameters indicated that the plant height, leaf number, leaf area, leaf thickness, number of branches, chlorophyll number, and stem diameter were not significantly different ($P>0.05$) under drought stress levels. However, the drought stress levels were significantly affected ($P<0.05$) on the biomass. As can be seen from Fig.1, the lowest plant height, leaf number, leaf area, leaf thickness, number of branches, and chlorophyll number observed on the W1 (40% FC) as compared to W2 (60% FC), W3 (80% FC), and W4 (100% FC-control).

The lowest growth obtained under W1 treatment while the highest of all the growth parameters obtained under W4 treatment except for the stem diameter and biomass obtained under W3 treatment. The W1 treatment as compared to W4-control reduced 8.39% plant height, 7.28% leaf number, 10.16% leaf area, 4.13% chlorophyll number, 3.63% leaf thickness, 3.33% stem diameter, 15.00% number of branches, and 18.37% biomass.

Phytochemical screening

Phytochemical screening of the leaves, stems, and fruits extracts of cultivated *F. deltoidea* (Table 1) under drought stress found that: (1) the leaves extract contained alkaloid, flavonoid, phenolic, and steroid; (2) the stems extract contained alkaloid, flavonoid, phenolic, tannin, steroid, coumarin, and carotenoid; (3) the fruits extract contained alkaloid, flavonoid, phenolic, steroid, and coumarin.

The total phenolic content (TPC)

The results of TPC on cultivated *F. deltoidea* under drought stress indicated that drought stress levels

significantly affected the total phenolic content of leaves, stems, and fruits of cultivated *F. deltoidea* (Table 2). The lowest value of TPC leaves (42.53 ± 0.02 µg GAE mg^{-1} extract), stems (38.27±0.00 µg GAE mg^{-1} extract), and fruits (3.70±0.01 µg GAE mg^{-1} extract) were obtained by W4 (100% FC-control) treatment.

Table 1. Phytochemical screening of leaves, stems, and fruits methanolic extracts of cultivated *F. deltoidea* under different drought stress levels.

Phytochemical compound	Treatments			
	W1	W2	W3	W4
Leaves Extract				
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Tannin	-	-	-	-
Steroid	+	+	+	+
Coumarin	-	-	-	-
Carotenoid	-	-	-	-
Saponin	-	-	-	-
Stems Extract				
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Tannin	+	+	+	+
Steroid	+	+	+	+
Coumarin	+	+	+	+
Carotenoid	+	+	+	+
Saponin	-	-	-	-
Fruits extract				
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Tannin	-	-	-	-
Steroid	+	+	+	+
Coumarin	+	+	+	+
Carotenoid	-	-	-	-
Saponin	-	-	-	-

Remarks: + = Presence of phytochemical compound; - = Absence of phytochemical compound. Drought stress levels; W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC.

The highest value of TPC leaves (64.37±0.02 µg GAE mg^{-1} extract), stems (74.07±0.001 µg GAE mg^{-1} extract), and fruits (24.07±0.001 µg GAE mg^{-1} extract) were obtained by W1 (40% FC) treatment and significantly different to the W2, W3, and W4 treatment. Drought stress treatment significantly increased the phenol content in leaves, stems, and fruits of cultivated *F. deltoidea*. The highest percentage increase of phenols was observed in W1 followed by W2 and W3 respectively as compared to W4-control. The stems extract has the highest total phenolic content as compared to the leaves and fruits extract.

The total flavonoid content (TFC)

Drought stress treatment enhanced the total flavonoid content of leaves, stems, and fruits extract of *F. deltoidea*.

The W1 produced the highest total flavonoid content on leaves, stems, and fruits extracts and significantly different from W2, W3, and W4 (Table 2).

Table 2. Effect of drought stress levels on total phenolic (TPC) and total flavonoid content (TFC) of cultivated *F. deltoidea*.

Plant parts	Treatments			
	W1	W2	W3	W4
	TPC (µg GAE/mg extract)			
Leaves	64.37±0.002[a]	50.58±0.003[b]	49.43±0.002[c]	42.53±0.001[d]
Stems	74.07±0.001[a]	69.14±0.006[b]	53.09±0.003[c]	38.27±0.002[d]
Fruits	24.07±0.001[a]	12.04±0.001[b]	4.63±0.001[c]	3.70±0.001[d]
	TFC (µg CE/mg extract)			
Leaves	397.44±0.007[a]	392.31±0.004[b]	228.21±0.017[c]	205.13±0.013[d]
Stems	162.00±0.002[a]	158.00±0.001[b]	154.00±0.008[c]	146.00±0.001[d]
Fruits	311.54±0.001[a]	246.15±0.004[b]	242.31±0.019[c]	184.62±0.013[d]

Note: W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC. Data are mean values ± SE (n=3). Mean values in the same rows followed by the same letter are not significantly different according to the DMRT at 95% confidence level.

The highest total flavonoid content found on the leaves extract (397.44±0.007 µg CE/mg extract) followed by fruits (311.54±0.001µg CE/mg extract) and stems (162.00±0.002 µg CE/mg extract) under W1 drought
stress.

Antioxidant activity

The drought stress significantly affected the antioxidant activity. Based on the IC_{50} value, the antioxidant activity of cultivated *F. deltoidea* extract (leaves, stems, fruits) was higher under severe drought stress-40% FC than 60%, 80%, and 100% FC level drought stress (Fig. 2).

The highest antioxidant activity found in the leaves extracts (72.47±0.050 μgmL^{-1}) under 40% FC followed by stems (157.71±0.036 μgmL^{-1}) and fruits (401.20±0.011 μgmL^{-1}). 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).

Discussion

The growth parameters

This study showed a rise in drought stress reduced plant height, leaf number, leaf area, leaf thickness, stem diameter, the number of branches, chlorophyll number, and biomass in cultivated *F. deltoidea*. The growth of the plant depends on cell expansion and enlargement. The most sensitive physiological aspect of a plant is water deficit condition that can reduce plant productivity and thus affects plant height, leaf number, stem diameter, and biomass.

The lower biomass of cultivated *F. deltoidea* showed in W1-40% FC treatment. Having a lower average of biomass under drought conditions can be interpreted as a response of plants towards drought stress.

This result supported by Manivannan *et al.* (2007) who found that the increased of the drought stress level, decreased the stem length and biomass of *Vigna unguiculata*. In addition, Specht *et al.* (2001) also reported that the stem length, biomass, and yield of soybean reduced as a result of drought stress treatment.

The plant height, biomass, and yield of parsley (*Petroselinum crispum*) decreased under drought stress (Petropoulos *et al.*, 2008). The higher plant produces antioxidant compounds and could limit biomass weight as a response to environmental stress such as drought stress (Shao *et al.*, 2008). The water deficits during the vegetative stage in *Dracocephalum moldavica* causing a decrease in the plant height, leaf area, and leaf number (Safikhani *et al.*, 2007).

Drought stress significantly decreased the chlorophyll content in *Helianthus annuus* L. (Kiani *et al.*, 2008), *Viccinum myrtillus* (Tahkokorpi *et al.*, 2007), and *Catharantus roseus* (Jaleel *et al.*, 2008). Recently, Duan *et al.* (2017) reported that the drought stress inhibited the seed germination and the growth of wheat seedlings.

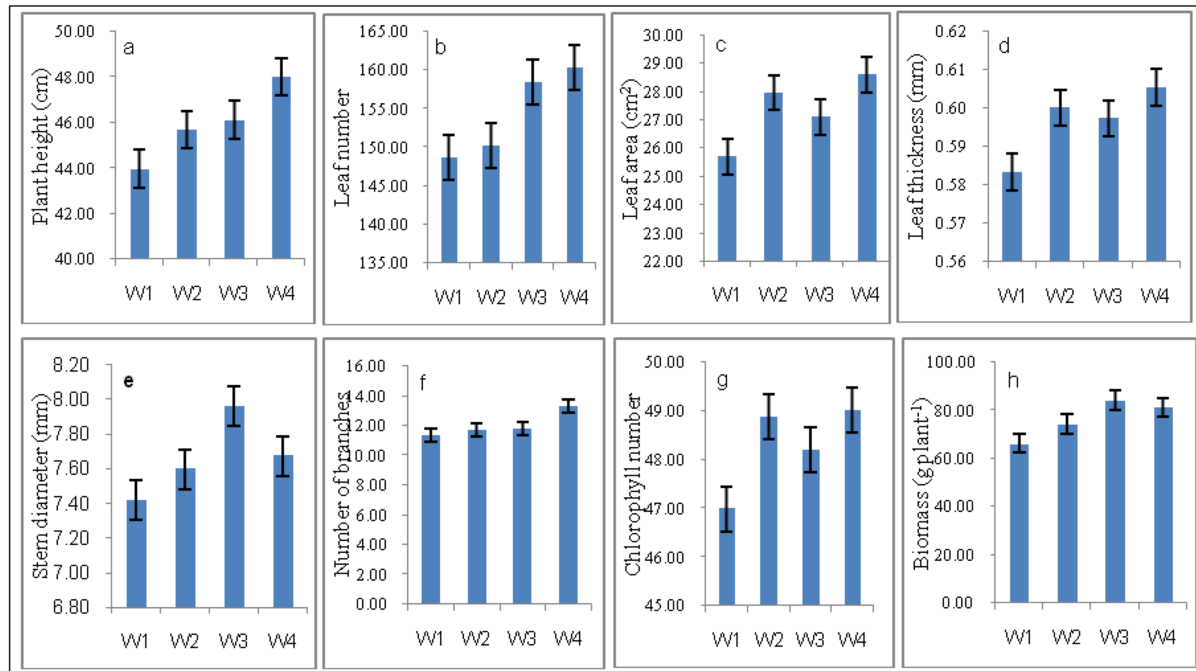

Fig. 1. Tabat barito (*Ficus deltoidea* Jack.) height (a), leaf number (b), leaf area (c) leaf thickness (d), stem diameter (e), number of branches (f), chlorophyll number (g), and biomass (h) under different drought stress levels. W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC. Data are mean values ± SE (n=3).

Phytochemical screening

Besides growth parameters, the phytochemical compounds that found in the leaves, stems, and fruits extract of cultivated *F. deltoidea* revealed that these plants have a potent as a medicinal capacity. Practically, the screening test revealed the presence of biologically important phytochemicals in cultivated *F. deltoidea* under drought stress is important to be done to find out their medicinal value. The results of this research may be useful in the process of cultivation of tabat barito (*F. deltoidea*). The phytochemical compound that contained in the leaves, stems, and fruits proves that the cultivated *F. deltoidea* under drought stress does not alter its usefulness as a medicinal plant. The highest phytochemical compound was found in extracts of stressed *F. deltoidea* in terms of total phenolic and flavonoid contents. In plants, accumulation of secondary metabolite stimulated as a response to biotic/abiotic constraints. These findings are in line with Al-Gabbiesh *et al.* (2015) stating that drought stress increased the production of secondary metabolites in spice and medicinal plant. The content of phenolics compound of leaves, stems, and the seed of *Arachis hypogaea* vary significantly increased under water stress (Aninbon *et al.*, 2016).

The production of phenolics in medicinal plants- *Silybummarianum* and *Achillea filipendulina* improved under drought stress. In addition, Ncib *et al.* (2018) reported that the amount of secondary metabolite in the root bark of *Rhustripartitum* and *Periploca laevigata* increased significantly under water deficit.

Total phenolic content (TPC) and total flavonoid content (TFC)

Our present study showed that the higher drought stress treatment on cultivated *F. deltoidea*, the higher total phenolic, and total flavonoid content produced.

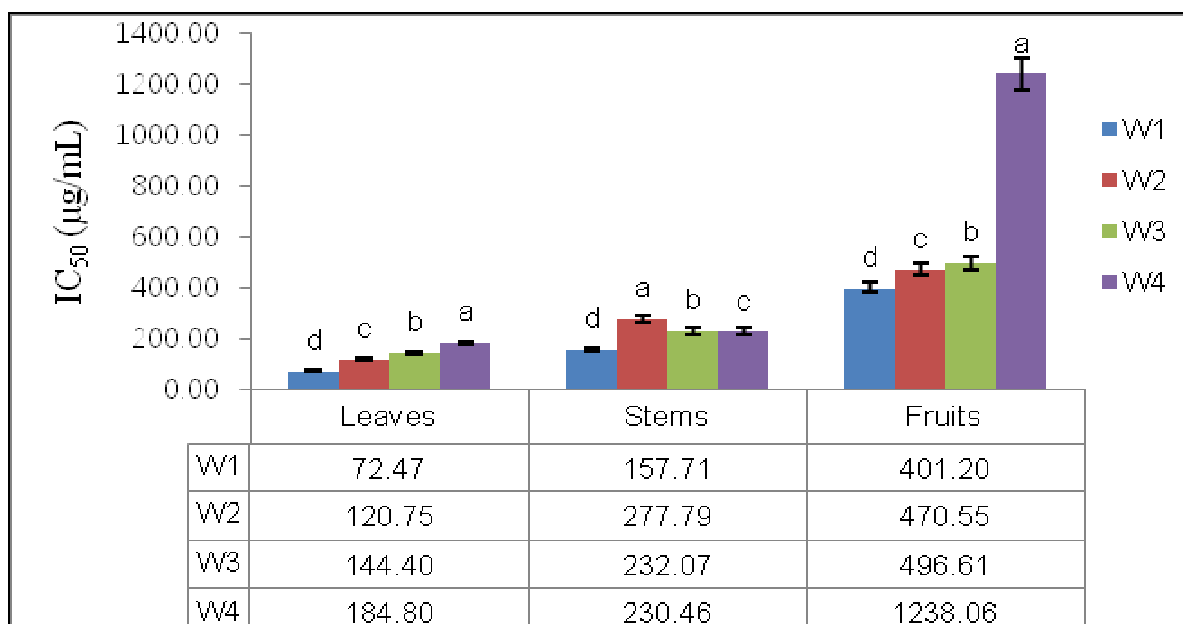

Fig. 2. Antioxidant activity based on IC_{50} values of leaves, stems, and fruits extract of cultivated *F. deltoidea* under different drought stress levels. W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC. Data are mean values ± SE (n=3). Mean values within each graph in figure followed by the same letter are not significantly different according to the DMRT at 95% confidence level.

The similar results were observed for the medicinal plant *Hypericum brasilience* under drought stress by Nacif and Mazzafera (2005).

The level of the phenolic compound was increased in the plant under drought stress. Phenol is one of the secondary metabolite compounds produced by plants when exposed to environmental stress. Zhu *et al.* (2009) found that phenolic content increased in *Bupleurum chinense* when plant exposes to drought conditions. Furthermore, Doaa and Nour (2012) reported that the total phenols in pepper leaves were significantly enhanced underwater conditions. The production of total phenols and flavonoids in *Labisia pumila* enhanced under drought stress (Jaafar *et al.*, 2012). Drought stress has the potential to increase pharmaceutical significant secondary compounds in plants (Selmar, 2008). The high level of phenol and flavonoid in *F. deltoidea* extract may help this plant to adapt drought stress. Thus the *F. deltoidea* could be considered as a tolerant plant to drought stress.

On cultivated *F. deltoidea*, the stems extract has the highest total phenolic on severe drought stress-W1 40% FC treatment followed by the leaves and fruits and the lowest on normal stress-W4 100% FC.

The leaves extract has the highest total flavonoid content as compared to stems and fruits. This finding is in line with Manurung *et al.* (2017[b]) stated that the leaves extract of the wild and cultivated *F. deltoidea* has higher total flavonoid content than the flavonoid in the stem.

Antioxidant activity

Our results revealed that the leaves extract has a higher antioxidant activity followed by stems and fruits obtained under W1 treatment. The increase in antioxidant activity was parallel with the increasing of drought stress levels. Antioxidants protect the cells from free radicals and therefore have been considered in several researches as a method to improve plant defense responses (Espinozaa *et al.*, 2013).

The total flavonoid had a positive correlation with the antioxidant activity in *Calamus tenuis* Roxb (Ahmed *et al.*, 2014). Methanolic leaves extract of *F. deltoidea* were able to scavenge DPPH free radicals with the low IC_{50} value (74.47 $mgmL^{-1}$) recorded for W1-40% FC treatment-the high drought stress.

The highest antioxidant activity of leaves, stems, and fruits methanolic extracts of *F. deltoidea* were obtained under W1 while the lower antioxidant activity was obtained under W4-100% FC. It means the higher drought stress the higher antioxidant activity has resulted. Our result is in line with the finding of Saeidnejad *et al.* (2013) and Bettaieb *et al.* (2012) who reported that under drought stress, there is an increase of antioxidant activity of medicinal plant *Bunium persicum* and *cuminum cyminum* seeds.

Conclusion

The investigation showed that drought stress affected *Ficus deltoidea* growth, phytochemical compound, total phenolic content, total flavonoid content, and antioxidant activity. Drought stress reduced the growth, whereas the drought stress treatment increased the total phenolic content, total flavonoid content, and antioxidant activity. The leaves, stems, and fruits of cultivated *F. deltoidea* have the varied secondary metabolite compound. The highest total phenolic, total flavonoid content, and antioxidant activity obtained from the highest drought stress treatment (W1 = 40% FC). These result showed that drought stress can be used to improve the secondary metabolite compound and antioxidant activity in cultivated *F. deltoidea*.

Declaration of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

Ahmed ZU, Bithi SS, Khan MMiR, Hossain MM, Sharmin S, Rony SR. 2014. Phytochemical screening, antioxidant extracts of Calamus tenuis Roxb and cytotoxic activity of fruit. Journal of Coastal Life Medicine **2,** 645–650. https://doi.org/10.12980/JCLM.2.201414D74

Al-gabbiesh A, Kleinwächter M, Selmar D. 2015. Influencing the contents of secondary metabolites in spice and medicinal plants by deliberately applying drought stress during their cultivation. Jordan Journal of Biological Science **8,** 1–10.

Aninbon C, Jogloy S, Vorasoot N, Patanothai A, Nuchadomrong S, Senawong T. 2016. Effect of end of season water deficit on phenolic compounds in peanut genotypes with different levels of resistance to drought. Food Chemistry **196,** 123–129. https://doi.org/10.1016/j.foodchem.2015.09.022

Arung ET, Kusuma IW, Christy EO, Shimizu K. 2009. Evaluation of medicinal plants from central Kalimantan for antimelanogenesis. Journal of Natural Medicine **63,** 473–480. https://doi.org/10.1007/s11418-009-0351-7

Azis N, Agarwal R, Mohd Ismail N, Ismail NH, Ahmad KMS, Radjeni Z, Singh HJ. 2017. Possible involvement of RAAS in the mechanism of anti-hypertensive effect of standardized aqueous ethanolic extract of Ficus deltoidea Kunstleri in spontaneously hypertensive rats. International Journal of Cardiology **249,** S6. https://doi.org/10.1016/j.ijcard.2017.09.042

Baloğlu MC, Kavas M, Aydin G, Öktem HA, Yücel AM. 2012. Antioxidative and physiological responses of two sunflower (Helianthus annuus) cultivars under PEG-mediated drought stress. Turkish Journal of Botany **36,** 707–714. https://doi.org/10.3906/bot-1111-20

Bettaieb-Rebey I, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B. 2012. Effect of drought on the biochemical composition and antioxidant activities of cumin (Cuminum cyminum L.) seeds. Industrial Crops and Products **36(1),** 238–245. https://doi.org/10.1016/j.indcrop.2011.09.013

Chung I, Kim J, Lim J, Yu C, Kim S, Hahn S. 2006. Comparison of resveratrol, SOD activity, phenolic compounds and free amino acids in Rehmannia glutinosa under temperature and water stress. Environmental and Experimental Botany **56,** 44–53.
https://doi.org/10.1016/j.envexpbot.2005.01.001

Das S, Bhattacharya SS. 2016. Plant secondary metabolites, in: Siddiqui, M.W., Bansal, V. (Eds.), their roles in stress ecophysiology. Canada Apple Academic Press, Oakville, p 1–38.

Doaa M, Nour KAM. 2012. Impact of salinity and biological and organic treatments on growth, yield and fruits quality of sweet pepper. Mansoura Journal of Plant Production **3,** 2883–2902.

Duan H, Zhu Y, Li J, Ding W, Wang H, Jiang L, Zhou Y. 2017. Effects of drought stress on growth and development of wheat seedlings. International Journal of Agriculture & Biology **19,** 1119-1124.
https://doi.org/10.101610.17957/IJAB/15.0393

Espinozaa A, Martína AS, López-Climentb M, Ruiz-Laraa S, Gómez-Cadenasb A, Casarettoa J. 2013. Engineered drought-induced biosynthesis of a-tocopherol alleviates stress-induced leaf damage in tobacco. Journal of Plant Physiology **170,** 1285–1294.
https://doi.org/10.1016/j.jplph.2013.04.004

Farahani HA, Valadabadi SA, Daneshian J, Khalvati MA. 2009. Evaluation changing of essential oil of balm (Melissa officinalis L.) under water deficit stress conditions. Journal of Medicinal Plants Research **3(5),** 329–333.

Geerts S, Raes D. 2009. Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. Agricultural Water Management **96,** 1275–1284.
https://doi.org/10.1016/j.agwat.2009.04.009

Gharibi S, Tabatabaei BES, Saeidi G, Goli SAH. 2016. Effect of drought stress on total phenolic, lipid peroxidation, and antioxidant activity of Achillea Species. Applied Biochemistry and Biotechnology **178(4),** 796-809.
https://doi.org/10.101610.1007/s12010-015-1909-3

Hakiman M, Maziah M. 2009. Nonenzymatic and enzymatic antioxidant activities in aqueous extract of different Ficus deltoidea accessions. Journal of Medicinal Plants Research **3(3),** 120–131.

Jaafar HZ, Ibrahim MH, Mohamad Fakri NF. 2012. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian kacip fatimah (Labisia pumila Benth). Molecules **17,** 7305–7322.
https://doi.org/10.3390/molecules17067305

Jaleel CA, Sankar B, Murali PV, Gomathinayagam M, Lakshmanan GMA, Panneerselvam R. 2008. Water deficit stress effects on reactive oxygen metabolism in Catharanthus roseus; impacts on ajmalicine accumulation. Colloids Surfaces B Biointerfaces **62,** 105–115.
https://doi.org/10.1016/j.colsurfb.2007.09.026

Khorasaninejad S, Mousavi A, Soltanloo H, Hemmati K. 2011. The effect of drought stress on growth parameters, essential oil yield and constituent of Peppermint Mentha piperita (L .). Journal of Medicinal Plants Research **5,** 5360–5365.

Kiani S, Poormohammed, Maury P, Sarrafi A, Grieu P. 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (Helianthus annuus L.) under well-watered and water-stressed conditions. Plant Science **175,** 565–573.
https://doi.org/10.1016/j.plantsci.2008.06.002

Kumari A, Sharma RA. 2015. Estimation of total phenol, flavonoid contents and DPPH free radical scavenging activity of Oxalis corniculata Linn. International Journal of Biological & Pharmaceutical Research **6,** 178–181.

Manivannan P, Jaleel CA, Kishorekumar A, Somasundaram R, Sridharan R, Panneerselvam R. 2007. Changes in antioxidant metabolism of Vigna unguiculata (L.) Walp. by propiconazole under water deficit stress. Colloids Surfaces B Biointerfaces **57,** 69–74. https://doi.org/10.1016/j.colsurfb.2007.01.004

Manurung H, Kustiawan W, Kusuma IW. Marjenah. 2017[a]. Total flavonoid content and antioxidant activity in leaves and stems extract of cultivated and wild tabat barito (Ficus deltoidea Jack). AIP Conference Proc. **1813** ed RA Nugroho (American Institute of Physics), p 020007, 1-6. https://doi.org/10.1063/1.4975945

Manurung H, Kustiawan W, Kusuma IW. Marjenah. 2017[b]. Total flavonoid content and antioxidant activity of tabat barito (Ficus deltoidea Jack) on different plant organs and ages. Jornal of Medicinal Plants Studies **5,** 120–125. http://www.plantsjournal.com/archives/2017/vol5issue6/PartB/5-6-23-951.pdf

Molyneaux P. 2004. The use of the stable free radical diphenyl picryl-hidrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal Science Technology **26(2),** 211–219. www.thaiscience.info/journals/Article/SONG/10462423.pdf

Morshedloo MR, Craker LE, Salami A, Nazeri V, Sang H, Maggi F. 2017. Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono- and sesquiterpene synthesis in two oregano (Origanum vulgare L.) subspecies. Plant Physiology and Biochemistry **111,** 119–128. https://doi.org/10.1016/j.plaphy.2016.11.023

Nacif de-AI, Mazzafera P. 2005. Effect of water and temperature stress on the content of active constituents of Hypericum brasiliense Choisy. Plant Physiology and Biochemistry **43,** 241–248. https://doi.org/10.1016/j.plaphy.2005.01.020

Ncib S, Boukhris M, Le E, Adesso S, Autore G, Marzocco S, Hanchi B. 2018. Effects of water deficit and rehydration on antioxidant and anti-inflammatory activities in methanolic root barks extracts of Rhus tripartitum and Periploca laevigata subsp. angustifolia. Industrial Crops and Products **111,** 353–359. https://doi.org/10.1016/j.indcrop.2017.10.034

Petropoulos SA, Daferera D, Polissiou MG, Passam HC. 2008. The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. Scientia Horticulturae **115,** 393–397. https://doi.org/10.1016/j.scienta.2007.10.008

Saeidnejad AH, Kafi M, Khazaei HR, Pessarakli M. 2013. Effects of drought stress on quantitative and qualitative yield and antioxidative activity of Bunium persicum. Turkish Journal of Botany **37,** 930–939. https://doi.org/10.3906/bot-1301-2

Safikhani FA, Sharifabad HH, Elah SSA, Ashourabadi ES, Seyenezhad SM, Abbaszadeh B. 2007. The effect of drought stress on percentage and yield of essential oil and physiological characteristics of Dracocephalum moldavica L. Iranian Journal of Medicinal and Aromatic Plants Research **23,** 86–99.

Selmar D. 2008. Potential of salt and drought stress to increase pharmaceutical significant secondary. Landbauforsch. - vTI Agriculture and Forestry Research **58,** 139–144. https://literatur.thuenen.de/digbib_extern/bitv/dk040469.pdf

Shao HB, Chu LY, Shao MA, Jaleel CA, Mi HM. 2008. Higher plant antioxidants and redox signaling under environmental stresses. Comptes Rendus Biologies **331,** 433–441. https://doi.org/10.1016/j.crvi.2008.03.011

Specht JE, Chase K, Macrander M, Graef GL, Chung J, Markwell JP, Germann M, Orf JH,

Lark KG. 2001. Soybean response to water : A QTL analysis of drought tolerance. Crop Science **41,** 493–509.

Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, Israf DA. 2008. Evaluation of the antinociceptive activity of Ficus deltoidea aqueous extract. Fitoterapia **79,** 557–561.
https://doi.org/10.1016/j.fitote.2008.06.005

Tahkokorpi M, Taulavuori K, Laine K, Taulavuori E. 2007. After-effects of drought-related winter stress in previous and current year stems of Vaccinium myrtillus L. Environmental and Experimental Botany **61,** 85–93.
https://doi.org/10.1016/j.envexpbot.2007.03.003

Zhu Z, Liang Z, Han R. 2009. Saikosaponin accumulation and antioxidative protection in drought-stressed Bupleurum chinense DC. Plants. Environmental and Experimental Botany **66,** 326–333.
https://doi.org/10.1016/j.envexpbot.2009.03.017