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

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Key words: Drought stress, Phytochemicals, Antioxidant activity, Ficus deltoidea.

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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), 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 (IC50 = 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 (IC₅₀ = 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 hav 32 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, biomen 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 phynolic 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 penper (Capsicum annuum) (Doaa and Nour, 2012) increased under drought stress. In addition, Nacif and Maggafera (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 reported 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 study by Misbah et al. (2013) evaluated that the eous extract and fraction of fruits F. deltoidea has antidiabetic and antioxidant properties. Manurung et al. (2017a) 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 (Azisat 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 fash leaves aqueous extract of *F. deltoidea* has non-enzymatic and enzymatic antioxidant activities.

In Kalimantan-Indonesia, the utilization 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 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 physicology 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 samples were weighed by using an electrical scale and dried in one electric oven at 105°C for 48 hours. The rate of 100% field capacity was measured following the equation.

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

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 leaf area, leaf thickness, stem diameter, the number leaf 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 open ried 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; Kolate, 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 seponin: 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 H2SO4 was then added slowly through the sides of the test tube to form a aver. 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, beiled 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 commarins. (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 faits 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 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. Folinciocalteu reagents: to one mL Folin-ciocalteu was added with 9 mL sedium carbonate 7.5% (7.5 mg of sodium carbonate dissolved in 100 L 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 or 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 neasured 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 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 (ATCT) method (Kumari and Sharma, 2015). One mg of methanolic extract was dissolved in 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 NaNO 31, 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 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
CE mg-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 antionizant 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 μ L each) was added to 467 μ L of ethanol and 500 μ L 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:

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

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, tanain, 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 \pm 0.02 μ g GAE mg⁻¹ extract), stems (38.27 \pm 0.00 μ g

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		Treatmen	ts	
_	W1	W2	W3	W4
	Leaves E	xtract		
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Tannin	-	-	-	-
Steroid	+	+	+	+
Coumarin	-	-	-	-
Carotenoid	-	-	-	-
Saponin	-	-	-	-
	Stems E	xtract		
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Phenolic	+	+	+	+
Tannin	+	+	+	+
Steroid	+	+	+	+
Coumarin	+	+	+	+
Carotenoid	+	+	+	+
Saponin	-	-	-	-
	Fruits ex	xtract		
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 leave $^{\circ}_{39}^{+}4.37\pm0.02~\mu g$ GAE mg⁻¹ extract), stems $(74.07\pm0.001~\mu g$ GAE mg⁻¹ extract), and fruits $(24.07\pm0.001~\mu g$ GAE mg⁻¹ extract) were obtained by W1 (40%~FC) treatment and significantly different to the W2, W3, and W4 treatment. Drought $^{\circ}_{35}$ by treatment significantly increased the phenol content in leaves, stems, and fruits of cultivated $^{\circ}_{5}$ 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	ts Treatments			
-	W1	W2	W3	W4
	TI	PC (μg GAE/mg extract)		
Leaves	64.37±0.002ª	50.58±0.003 ^b	49.43±0.002°	42.53±0.001 ^d
Stems	74.07±0.001 ^a	69.14±0.006 ^b	53.09±0.003 ^c	38.27±0.002d
Fruits	24.07±0.001 ^a	12.04±0.001 ^b	4.63±0.001°	3.70±0.001 ^d
	T	FC (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±0.002ª	158.00±0.001 ^b	154.00±0.008c	146.00±0.001
Fruits 94	311.54±0.001ª	246.15±0.004 ^b	242.31±0.019 ^c	184.62±0.013 ^d

Values: 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 \pm 0.007 μ g CE/mg extract) followed by fruits (311.54 \pm 0.001 μ g CE/mg extract) and stems (162.00 \pm 0.002 μ g CE/mg extract) under W1 drought stress.

Antioxidant activity

The drought stress significantly affected antioxidant activity. Based on the IC_{50} value, the antioxidant activity of cultivated F. deligidea 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 µgmL¹) under 40% FC followed by stems (157.71±0.036 µgmL¹) and fruits (401.20±0.011 µgmL¹). The strength of the antioxidant activity categorized as strong (IC₅₀< 50 ppm), active (IC₅₀ = 50-100 ppm), moderate (IC₅₀ = 101-150 ppm), and weak (IC₅₀ = 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 yill of parsley (Petroselinum crispum) decreased under drought stress (Petropoulos et al., 2008). The higher plant produces antioxidant compounds and could limit romass weight as a response to environmental stress such as drought stress (Shao et al., 2008). The water deficits during the vegetative stage in the plant height, leaf area, and leaf number (Safikhani et al., 2007).

Drought stages significantly decreased the chlorophyll content in Helianthus annuus L. (Kiani et al., 2008), Viccinum myrtillus (Talkokorpi 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.

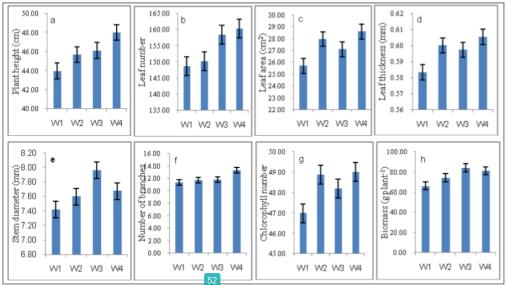


Fig. 1. Tabat barito (*Ficus deltoidea* Jack.) height (a), leaf number (b), leaf area (c) leaf thickness (d), stem diameter (e), number of branches (f), chlorophyll number (g), and biomass (h) under different drought stress levels. W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC. Data are mean values \pm 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 rought stress increased the production of secondary metabolites in spice and medicinal plant. The content of phenolics compound of leaves, stems, and the seed Arachis hypogaea vary significantly increased under water stress (Aninbon et al., 2016).

The production of phenolics in medicinal plants-Silybummarianum and 76 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 75 ss treatment on cultivated *F. deltoidea*, the higher total phenolic, and total flavonoid content produced.

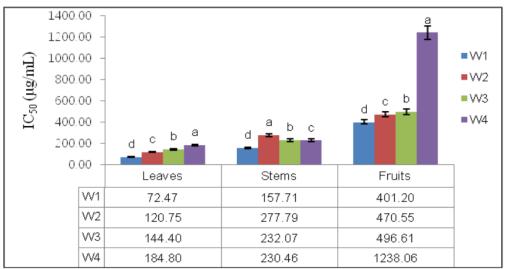


Fig. 2. Antioxidant activity based on IC₅₀ values of leaves, stems, and fruits extract of cultivated F. deltailed under different drought stress levels. W1= 40% FC; W2 = 60% FC; W3 = 80% FC; W4 = 100% FC. Data are mean values \pm SE (n=3). Mean values within each graph in figure followed by the same 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 pificantly enhanced underwater conditions. The production of total phenols and flavonoids in Labisia pumila enhanced under drought stress (June 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 ant 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. (2017b) 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.*, 2012) Methanolic leaves extract of *F. deltoidea* were able to scavenge DPPH free radicals with the low IC₅₀ value (74.47 mgmL⁻¹) recorded for W1-40% FC treatment-the high drought stress.

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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 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 deltoidea growth, phytochemical compound, total phenolic content, total flavonoid content, and antioxidant activity. Drought stress reduced the growth, where 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 variable 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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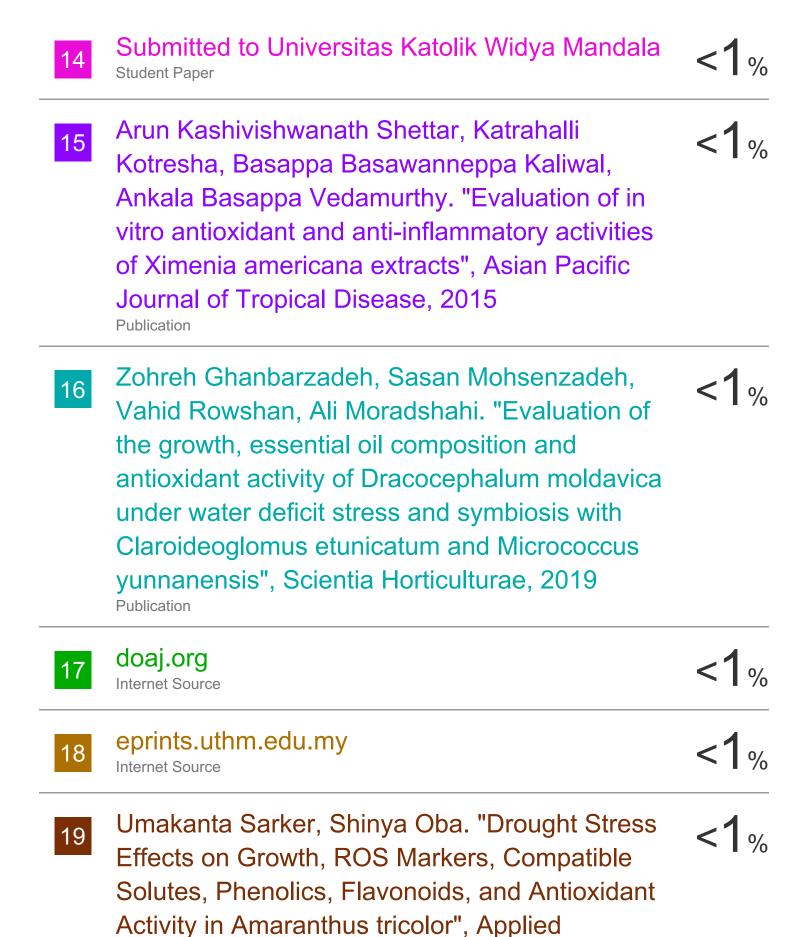
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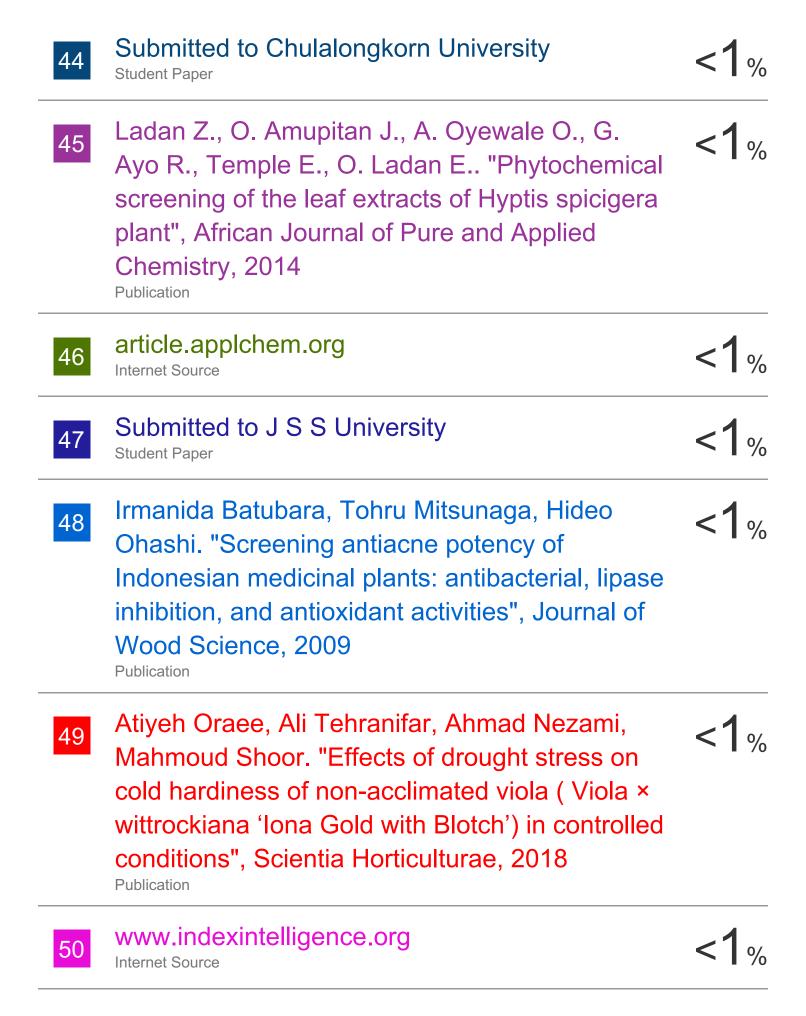
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