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Growth and ursolic acid content of pearl grass (*Hedyotis corymbosa*) on variations in water availability

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Abstract. *Anam K, Mudyantini, Rakhmawati R. 2017. Growth and ursolic acid content of pearl grass (*Hedyotis corymbosa*) on variations in water availability. Cell Biol Dev 1: 55-62.* The purpose of this study is to determine how different water sources affect the growth and levels of ursolic acid in pearl grass (*Hedyotis corymbosa* (L.) Lam.). A factorial, completely randomized design (CRD) with a single factor of five replications each was used for the experiment. Water stress conditions were created by providing different levels of water availability in the growing media, namely 40, 60, 80, and 100% field capacity (control). The plants were given the treatment for 8 weeks. Plant growth, including the number of leaves, fresh and dry weight, and plant ursolic acid content, were all measured in this study. The data were analyzed using analysis of variance (ANOVA), and the DMRT test at the 5% test level was used to determine the significant difference between treatments. The results showed that the water availability treatment significantly affected the number of leaves, fresh weight, and dry weight, but it did not affect the ursolic acid content in plants. The treatment with the lowest water availability (40% KL) produced low yields on growth parameters and ursolic acid levels. Ursolic acid levels were lowest under drought stress conditions (40% KL), while they were highest under excess water conditions (100% KL).

Keywords: Growth, pearl grass, ursolic acid, water availability

INTRODUCTION

The desire to reconnect with nature is increasingly dominating the community at this time. Synthetic treatment is deemed too costly and has serious side effects. Furthermore, the monetary crisis that has gripped Indonesia since mid-1997 has caused the price of medicines to skyrocket, rendering them unaffordable to the general public (Yuliani 2001). Traditional medicine is generally considered to be safer than modern medicine. This is because traditional medicine has fewer side effects than modern medicine (Sari 2006). The growing use of traditional medicines necessitates the development of more genuine traditional medicines in terms of health, economic potential, and community welfare (Yuliani 2001).

As one of the mega biodiversity countries, Indonesia is well-known for its medicinal plant reserves. Around 9,600 of the approximately 30,000 plant species in Indonesia have medicinal properties. Some 283 were identified as important medicinal plants for the traditional medicine industry (Kusuma and Zaky 2005; Sukandar 2006). The Indonesian people have long known and used medicinal plants to treat health issues. Knowledge of medicinal plants has been passed down from generation to generation based on experience and skills (Sari 2006). The benefits of using traditional medicines that are felt directly by the community are the ease with which they can be obtained, and the raw materials can be planted in their yards, inexpensive, and mixed themselves (Zein 2005).

Pearl grass (*Hedyotis corymbosa* (L.) Lam.) is one of the plants with medicinal potential. *H. corymbosa* is a plant that grows in moist soil on the sides of roads, yards, and ditch edges. The grass grows shady and scattered, is rather weak, grows 15-50 cm tall, and has many branches. The stem is angular, the leaves are opposite each other, the leaf stalk is short/almost sitting, the leaf length is 2-5 cm, the tip is pointed, and there is one leaf bone in the center. Short hairs cover the tips of the leaves. Flowers emerge from the leaf axils in the form of 2-5 compound flowers, with flower stalks (mother) that are hard like wire and 5-10 mm long. The fruit has been constructed, and the ends have been cracked (Ipteknet 2005).

Ursolic acid is one of the secondary metabolites found in *H. corymbosa*. This compound has anti-tumor (Yamaguchi et al. 2008), anti-inflammatory (Baricevic et al. 2001), hepatoprotective, anti-ulcer, anti-hyperlipidemic, and anti-microbial properties (Pendleton 2009). Hsu (1998) investigated three *H. corymbosa* compounds: ursolic, oleanolic, and geniposidic acids. As a result, ursolic acid and oleanolic acid can inhibit hep-2B cell growth and subcutaneous tumor growth.

Hedyotis corymbosa is commonly used in traditional medicine to treat cervical, stomach, breast, rectum, nasopharynx, fibrosarcoma, and lymphosarcoma cancers (Ipteknet, 2005). However, this plant has not been widely cultivated. Because this plant is frequently neglected and considered a weed, its population is declining because it is removed when the yard is cleaned. As a result, studies to assess the growth of this plant are required, given its high potential as a medicinal plant.

The growth and ursolic acid content of *H. corymbosa* have received little attention. When a plant is stressed, its

growth and secondary metabolite content increase. Water availability is an important environmental factor influencing plant secondary metabolite growth and content.

The purpose of this research is to determine the growth and ursolic acid content of *H. corymbosa* plants under various water availability conditions. Optimally treatment can be used in the field to boost productivity and secondary metabolite content.

MATERIALS AND METHODS

Materials

The main materials used are pearl grass seeds (*Hedyotis corymbosa* (L.) Lam.). of uniform age.

Research design

This study used a completely randomized design (CRD), with each treatment with 5 replications. The treatment of water availability (A) is as follows:

A_{100} = control (100% field capacity)
A_{80} = 80% field capacity
A_{60} = 60% field capacity
A_{40} = 40% field capacity

Procedure

Preparation

The seedling was carried out in perforated plastic tubs. The media used was a combination of soil and manure with a ratio of 2:1. In the media, an indentation is made 1-1.5 cm deep, then the seeds are inserted into it and covered with media. Watering is done 2 times every day. After the seeds grow about 4-5 cm, they are transferred in polybags with media like during seed nursery. Plants are grown in polybags for 2 weeks.

Determination of field capacity

The drained planting media mixture is weighed one kilogram in a perforated polybag at the bottom. The polybag was then watered until the water stopped dripping from the bottom hole, allowing the volume of water used for watering and its field capacity to be calculated. The following formula is used to calculate field capacity:

KL= (Weight of soil + polybag + water) - (Weight of soil + polybag) (Patoni 2000).

Treatment:

Plants were treated with various variations of water availability, including 100, 80, 60, and 40% of field capacity, by watering every 2 days.

Cultivation:

The cultivation time was 2 months at the Green House of the Faculty of Agriculture.

Growth observation:

The number of leaves: The number of leaves that appear was observed every 1 week until harvest. *Fresh weight*: Fresh weight of plants was weighed after the plants were treated with different water availability for 2 months. *Dry weight*: The plants were dried in an oven at 60ºC until dry and weighed.

Extraction

After being treated, *H. corymbosa* plants are dried in an oven to produce dry Simplicia, then powdered with a blender and sieved with a specific size sieve to produce dry powder, then macerated with methanol and allowed to stand for 24 hours. Maceration was performed three times. The maceration results were then filtered and dried to obtain methanol extract.

Thin layer chromatography

For comparison, each extract was spotted on the same TLC plate as standard ursolic acid. The mobile phase of petroleum ether: ethyl acetate (4:1 v/v), was then used to elute the sample (Srinivasan et al. 2008). The cerium (IV) sulfate reagent was used to detect the presence of organic compounds, while Lieberman-Burchard (LB) reagents were used to detect the presence of ursolic acid.

Determination of ursolic acid levels

The UV-vis spectrophotometer method was used, and the following procedures were followed:

Preparation of a standard ursolic acid solution standard curve: A concentration series is created by dissolving 10 mg of standard ursolic acid in 50 mL of sulfuric acid. The absorbance of each solution was measured with a wavelength of 310 nm to create a standard curve (Murav'ev et al. 1972). The linear regression line equation, generally formulated as $y = bx + a$, is searched using the standard solution content data as the x-axis and the absorbance value as the y-axis.

Ursolic acid concentration determination in the sample: Take 5 mg of the methanol extract sample and dissolve it in 10 mL of sulfuric acid at a series of different levels. A UV-vis spectrophotometer set to 310 nm was used to measure the absorbance of each sample solution. Five replications are performed.

Level calculation

The ursolic acid content of the sample is calculated by entering the absorbance value of the sample into the equation $y = bx + a$ obtained from the standard curve. Then the results of the ursolic acid content are converted into units of mg/g dry weight using the formula:

$$R = \frac{S \times V}{B}$$

Where; R: ursolic acid levels (mg/g); S: ursolic acid levels of spectrophotometric result sample (mg/L) and B: powder weight (g) (Hary 1998).

Data analysis

Quantitative data on the number of leaves, fresh weight, and dry weight of plants and levels of ursolic acid were analyzed by Anova; if there was a significant difference between treatments, then DMRT was carried out at test level 5%.

RESULTS AND DISCUSSION

Growth

Growth is defined as an increase in plant material. The whole process chemically changes these raw materials and adds them to the plant (Goldsworthy and Fisher 1992). According to Sitompul and Guritno (1995), growth is a process in plant life that causes changes in plant size and determines plant yields.

Three events occur as part of the growth and development process: (i) cell division, in which one adult cell divides into two separate cells; (ii) cell enlargement, in which one or both daughter cells increase in volume; and (iii) cell differentiation, in which cells that have already reached the volume become specialized. Cell division does not increase size; rather, it is the products of cell division that grow and cause growth. The expansion of plant body size is a real result of an increase in the size of cell parts caused by cell growth (Sitompul and Guritno 1995). The growth parameters observed in this study were the plant's number of leaves, fresh weight, and dry weight.

Number of leaves

Because leaves are sensitive to environmental changes, they are one of the parameters that can be observed. Because leaves are photosynthetic organs, they play a critical role in plant growth. Plant leaves, in general, are the site of carbohydrate synthesis. As a result, leaf observation is critical as a growth indicator and as supporting data to explain the growth process that occurs (Sitompul and Guritno 1995).

The analysis of variance results revealed that treatments with water availability of 40, 60, 80, and 100% field capacity (KL) significantly affected the number of leaves of *H. corymbosa* plants. Table 1 shows data on the average number of leaves on *H. corymbosa* plants with varying water availability over two months.

Figure 1 shows that the 100% KL water availability treatment had the most leaves, while the 40% KL treatment had the fewest. As can be seen, water availability of 100% KL is more optimal in increasing the number of leaves than water availability of 40%, 60%, and 80% KL. Figure 1 depicts a graph of the average number of *H. corymbosa* leaves for two months with varying water availability.

The highest number of leaves (409.2 strands) was found in 100% KL water availability. Photosynthesis is generally used for the growth of photosynthesis-active organs in conditions of abundant water. The fewest number of leaves discovered at 40% KL water availability was 180.4 strands. This is due to a lack of water availability in the soil, which causes plants to be thirsty and interferes with plant growth. Lack of water can reduce the photosynthesis rate, owing to stomata guard cells' turgidity. When there is a lack of water, the turgidity of the guard cells decreases, causing the stomata to close (Lakitan 1995).

Figure 2 depicts a graph of the average number of leaves on *H. corymbosa* plants every week for two months with varying water availability. Figure 2 depicts the total number of leaves in each treatment. From the first week to the end of the treatment, the number of leaves on *H. corymbosa* plants increased every week. Water stress decreases the water potential in plant cells. The decrease in water potential influences changes in plant hormone concentrations, particularly the hormone ABA (abscisic acid). When there is a lack of water, the ABA content of the leaves increases before the stomata close. The accumulation of ABA stimulates the flow of K+ ions, which causes water to exit the guard cells, lowering turgor pressure and causing stomatal closure. Closing these stomata prevents CO_2 uptake, which is required for carbohydrate synthesis. Closure of stomata, on the other hand, is beneficial because it can reduce the transpiration rate, thereby reducing plant water loss.

Table 1. The average number of *H. corymbosa* leaves for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average number of leaves (strand)	180.4[a]	278[b]	307.6[b]	409.2[c]

Notes: The numbers followed by the same letter were not significantly different at the 5% DMRT test level

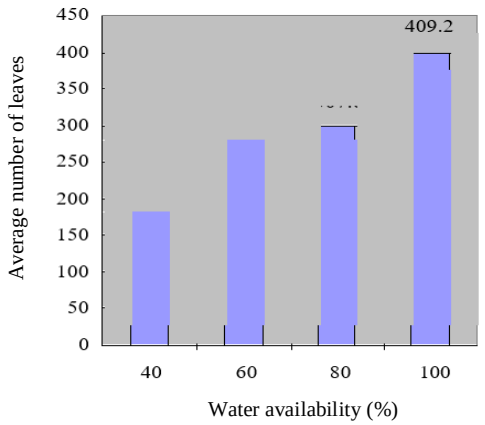

Figure 1. The average number of *H. corymbosa* leaves for 2 months with different water availability

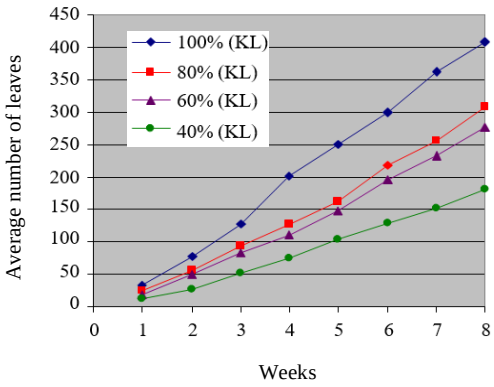

Figure 2. The average number of leaves of *H. corymbosa* plants every week for 2 months with different water availability

Plant growth is linked to cell division and enlargement. Auxin is a growth hormone that encourages cell growth and elongation (cell enlargement). Auxin is transported from cell to cell polarly in the stem, particularly in the basipetal. This auxin transport requires energy, which will be hampered if ATP synthesis is inhibited or there is a lack of oxygen (Anggarwulan and Solichatun 2001). When there is a lack of water, the photosynthesis rate slows, inhibiting ATP synthesis. This ATP synthesis inhibition will prevent auxin distribution, thereby inhibiting cell elongation and elongation. Plant growth will be slowed if cell elongation and elongation are inhibited.

Cell division can also cause a reduction in the number of leaves in plants (Abdalla and El-Khoshiban 2007). Cytokinins are hormones that promote cell division. Cytokinin levels will generally decrease during water stress (Pospisilova et al. 2000). Low cytokinin levels can inhibit cell division, resulting in a reduction in the number of leaves. According to Nautiyal et al. (1994), the number of leaves in three plant species, *Eucalyptus hybrid* (*E. camaldulensis* Dehnh. *x E. tereticornis* Sm.), *Casuarina equisetifolia* L., and *Melia azedarach* L., decreased as drought stress increased. Greitner et al. (1994) discovered that drought stress reduced the number of leaves on *Populus tremuloides* Michx.

Fresh weight

Plant biomass (weight) is the most commonly used metric for describing and studying plant growth. This is because the estimated plant weight is relatively easy to measure and represents an integration of almost all previous events encountered by the plant (Sitompul and Guritno 1995). The fresh weight of the plant reflects the plant's metabolic activity, and its value is influenced by the water content of the tissue, nutrients, and metabolic products. Weight gain is frequently determined by harvesting the entire plant or the desired part and quickly weighing it before too much water has evaporated from the material; this is known as fresh mass (Salisbury and Ross 1995). Table 2 displays the average fresh weight of *H. corymbosa* plants over two months with varying water availability.

Table 2 shows that differences in water availability result in significantly different fresh weights of plants. The highest average value of fresh plant weight was found at 100% KL, and the lowest was found at 40% KL. According to the findings of this study, lower levels of water availability result in lower plant fresh weight, while higher levels of water availability result in higher plant fresh weight.

The 40% KL water availability treatment had the lowest fresh weight was 1.25 g. This is due to a lack of water availability in the soil, which causes plants to be dehydrated, interfering with plant metabolism. According to Wilkinson (1994), a lack of water directly affects plant vegetative growth. Turgor stress governs this process in plants. Turgidity tension loss can halt cell growth (multiplication and enlargement), resulting in stunted plant growth. Figure 3 depicts a graph of the average fresh weight of *H. corymbosa* plants over two months with varying water availability.

A lack of water will cause disruptions in cell metabolism, including photosynthesis. Photosynthesis will be hampered if the main ingredient, water, is only available in limited quantities, resulting in lower photosynthesis results. Photosynthate produced will also be hampered in its circulation to all parts of the plant, reducing plant weight (Harjadi and Yahya 1988). Drought stress reduced the wet and dry weight of sesame (*Sesamum indicum* L.) plants, according to Fazeli et al. (2006).

Dry weight

According to Lakitan (1995), plant dry weight reflects the accumulation of organic compounds successfully synthesized by plants from inorganic compounds, particularly water and CO_2. The dry weight of the plant is obtained by drying the fresh weight of the plant to remove the water content in the plant; however, the fresh weight of the plant does not always determine the dry weight of the plant. The Table 3 shows the average dry weight of *H. corymbosa* plants after two months of different water availability.

Table 2. The average fresh weight of *H. corymbosa* plants for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average fresh weight (g)	1.25[a]	3.76[b]	4.47[b]	6.34[c]

Notes: The numbers followed by the same letter are not significantly different at the 5% DMRT test level

Table 3. The average dry weight of *H. corymbosa* plants for 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
The average dry weight (g)	0.26[a]	1.19[b]	1.59[b]	2.29[c]

Notes: the numbers followed by the same letter are not significantly different at the 5% DMRT test level

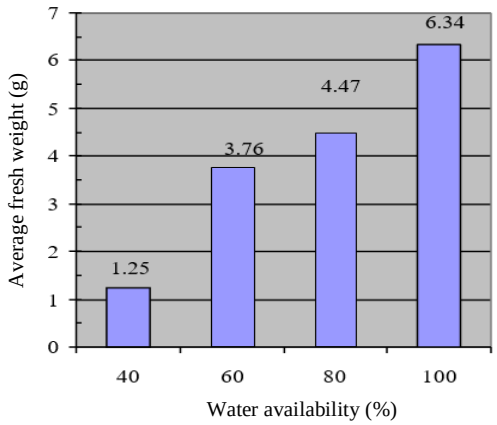

Figure 3. The average fresh weight of *H. corymbosa* plants for 2 months with different water availability

The analysis of variance revealed that treatments with water availability of 40, 60, 80, and 100% KL significantly affected the dry weight of *H. corymbosa* plants. The highest dry weight was found at 100% KL water availability and the lowest at 40% KL water availability.

The 40% KL water availability treatment had the lowest plant dry weight was 0.26 g. (Figure 4). Water scarcity can cause stomatal closure, reducing CO_2 uptake and resulting in stunted growth and reduced dry weight (Gardner et al. 1991). Water affected dry weight, according to Fitter and Hay (1998). This is related to the photosynthesis process. Photosynthesis accounts for the majority of plant dry weight. Figure 4 depicts the average dry weight of *H. corymbosa* plants over two months with varying water availability.

When there is a lack of water, the concentration of ABA hormone in plants increases, the increase in ABA hormone concentration will cause stomata to close, reducing CO_2 uptake for photosynthesis and, as a result, the rate of photosynthesis will decrease. According to Gardner et al. (1991), the reduced rate of photosynthesis caused by water stress occurs because the leaves formed under these conditions are inhibited by cell enlargement, resulting in smaller leaves than plants growing under normal conditions. This entails decreasing light absorption, thereby decreasing photosynthesis ability. The reduced rate of photosynthesis reduces the synthesis of body structures and food reserves, reducing dry weight. Drought stress reduced leaf height, length, dry weight, and leaf area in *Cymbopogon nardus* (L.) Rendle and *C. pendulus* (Nees ex Steud.) W.Watson, according to Sangwan et al. (1994). According to Solichatun et al. (2005), low water availability reduces the dry weight of Javanese ginseng (*Talinum paniculatum* (Jacq.) Gaertn.) plants.

Ursolic acid compounds

One of the chemical compounds found in *H. corymbosa* plants is ursolic acid. This compound is a pentacyclic triterpenoid compound found naturally in most herbaceous and fruiting plants (Pendleton 2009). These compounds have anti-tumor (Hah et al. 1992; Yamaguchi et al. 2008) and anti-inflammatory (Baricevic et al. 2001) effects, as well as hepatoprotective, anti-ulcer, anti-microbial, anti-hyperlipidemic, and anti-virus properties (Pendleton 2009).

Detection of ursolic acid compounds

Extraction is the first step in obtaining a compound from a sample or material. Before extraction, the harvested plants were dried in a 60°C oven and blended to obtain a dry powder. Extraction is the process of transferring or withdrawing the mass of the active substance in the cell; the filtered fluid will penetrate the cell wall and enter the active substance-containing cell cavity, causing the active substance to dissolve. Organic solvent extraction methods such as maceration, percolation, and soxhletation are used, as are water extraction methods such as infusion, decocted, and stem distillation (Silva et al. 1998).

The maceration extraction method was used in this study. Maceration is a straightforward extraction method. Maceration is derived from the Latin word macerase, which means to soften. To soften the cell structure, Simplicia powder with the desired fineness can be immersed in the liquid filter, and substances easily soluble in the liquid filter will be pulled out of the cell (Ansel 1989). Methanol can be used to extract ursolic acid compounds, according to Hamzah and Lajis (1998), so this study uses methanol to extract ursolic acid compounds. The dry Simplicia powder was macerated in methanol for 24 hours, occasionally stirring. The stirring process allows the liquid to penetrate the cell wall and filter out the compounds. The extraction process was repeated three times before filtering the juice and residue. The extracted extract was dried to produce a thick methanol extract.

Ursolic acid compounds were detected in a methanol extract of the *H. corymbosa* plant. TLC was used to detect the presence of these compounds, with petroleum ether as the mobile phase: ethyl acetate (4: 1 v/v) (Srinivasan et al. 2008), and standard ursolic acid as a comparison. Spray detection was also performed using cerium (IV) sulfate and Liebermann-Burchard (LB). The color reagent cerium (IV) sulfate generally is used to determine the presence of organic compounds; if organic compounds are present, the color of the spots will change. The LB color reagent is used to detect the presence of triterpenoid compounds; if triterpenoid compounds are present, the spots will turn green to blue (Harborne 1987).

Figure 5 depicts the chromatogram of the *H. corymbosa* plant methanol extract. The detection of the chromatogram with UV_{254} in Figure 5 shows the presence of attenuation, which is indicated by the presence of several dark spots. The presence of dark spots indicates the presence of a compound. UV_{366} detection revealed three fluorescent spots, one reddish (Rf 0.65) and two purple (Rf 0.13 and 0.41). This indicates that the compound has a long conjugated double bond and can fluoresce when exposed to long-wave UV irradiation. Because the chemical structure of these compounds did not contain conjugated double bonds, detection with UV_{254} and UV_{366} revealed no spots of ursolic acid compounds.

Chromatograms sprayed with LB color reagent yielded bluish spots with an Rf value of 0.35 on standard ursolic acid compounds and the same Rf value on each plant extract sample (Figure 5). It can be concluded that ursolic acid compounds are present in the methanol extract of the *H. corymbosa* plant.

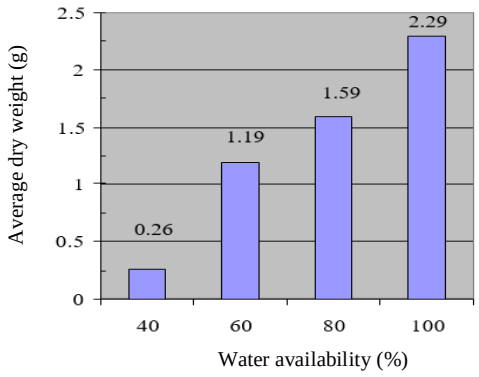

Figure 4. The average dry weight of *H. corymbosa* plants for 2 months with different water availability

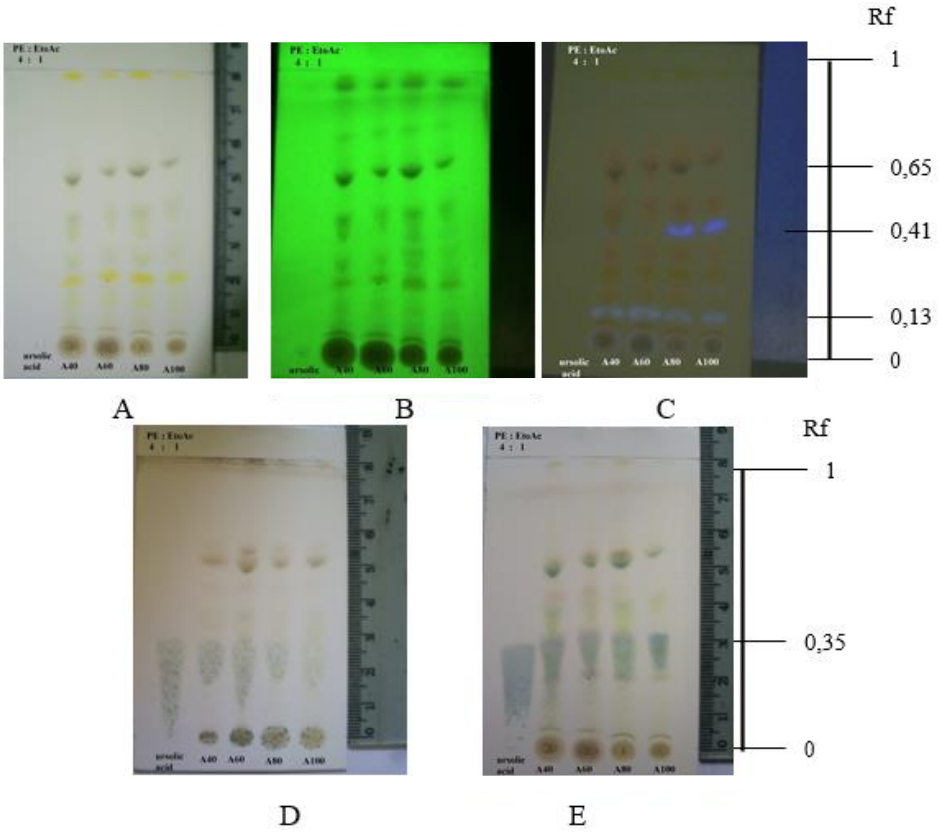

Figure 5. Chromatogram of *H. corymbosa* methanol extract with standard ursolic acid as a comparison with the detection of (A) visible light, (B) UV_{254}, (C) UV_{366}, (D) cerium (IV) sulfate, and (E) liebermann burchard Stationary phase: silica gel GF_{254}. Mobile phase: petroleum ether: ethyl acetate (4 : 1 v/v)

Ursolic acid compound levels

The analysis of variance revealed that treatments with water availability of 40, 60, 80, and 100% KL had no significant effect on ursolic acid compound levels in *H. corymbosa* plants. Table 4 shows the average levels of ursolic acid compounds in *H. corymbosa* plants after being given different water availability for two months.

The highest concentrations of ursolic acid compounds were found in 100% KL water availability and the lowest in 40% KL water availability. The lowest ursolic acid compound levels were found at 40% KL water availability, which was 37.96 mg/g dry weight (Figure 6). This occurs because the plants are deprived of water in these conditions. Because the turgidity of stomata guard cells decreases when there is a lack of water, photosynthesis can be slowed. As a result, the stomata close (Lakitan 1995). Closure of stomata in most species due to a lack of water in the leaves reduces the rate of CO_2 absorption and, as a result, the rate of photosynthesis (Goldsworthy and Fisher 1995). Figure 6 depicts a graph of the average levels of ursolic acid compounds in *H. corymbosa* plants over two months with varying water availability.

The slower rate of photosynthesis results in lower photosynthetic yields, which reduces the formation of ursolic acid compounds. Ursolic acid compounds are created through the glycolysis process, in which glucose molecules produced by photosynthesis are converted into pyruvate and acetyl-CoA.

Table 4. Average levels of ursolic acid compounds in *H. corymbosa* plants after 2 months with different water availability

Treatment	Water availability (%)			
	40	60	80	100
Average levels of ursolic acid (mg)	37.96	43.30	48.45	75.34

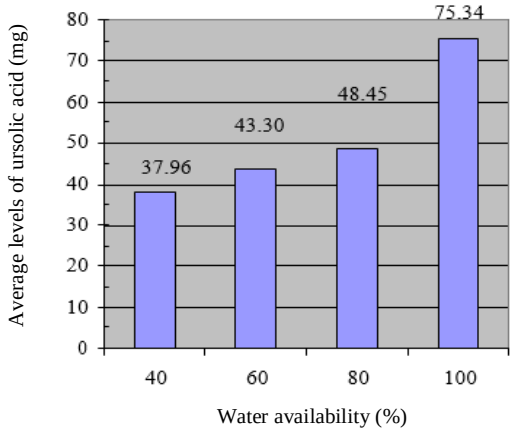

Figure 6. Average levels of the ursolic acid compound in *H. corymbosa* plants for 2 months with different water availability

Glycolysis, the first stage of carbon metabolism, is a series of reactions that convert hexose sugars (usually glucose) into pyruvic acid. Generally, glycolysis is divided into two stages: I A series of reactions that convert various forms of glucose and fructose from carbohydrate reserves to fructose-1,6-bisphosphate. (ii) Fructose-1,6-bisphosphate (FBP) is converted further into pyruvate (Anggarwulan and Solichatun 2001).

In addition, the pyruvate produced by glycolysis will be converted into acetyl-CoA via an oxidative decarboxylation process. Acetyl-CoA is converted into mevalonic acid, a precursor in the formation of ursolic acid compounds; however, if the raw material for the formation of mevalonic acid, namely glucose, is reduced, the formation of ursolic acid compounds is inhibited.

Furthermore, the concentration of the hormone ABA in leaves and fruit increased under water stress conditions. ABA is a 15-carbon sesquiterpene synthesized in chloroplasts and other plastids via the mevalonate pathway. The first reactions in the synthesis of ABA are identical to those of other isoprenoids like gibberellins, sterols, and carotenoids. A small amount of ABA is produced in chloroplasts by the breakdown of violaxanthin, a xanthophyll carotenoid that is converted to ABA during their metabolism (Anggarwulan and Solichatun 2001).

Mevalonic acid is the starting point for both ABA and ursolic acid. MVA kinase phosphorylates mevalonic acid to form mevalonic acid-5-phosphoric acid (MVAP), which is then phosphorylated by MVAP kinase to form mevalonic acid-5-diphosphate (MVAPP). Decarboxylation of MVAPP produces isopentenyl diphosphate (IPP), a precursor compound in forming various terpenoid compounds. Terpenoids are formed when IPP and its isomer dimethyl allyl diphosphate (DMAPP) combine to form larger molecules (Taiz and Zeiger 1998; Croteau et al. 2000). Geranyl transferase catalyzes the reaction of IPP and DMAPP to form geranyl pyrophosphate and farnesylpyrophosphate (Ngan 2005). Farnesyl-pyrophosphate is a precursor of sesquiterpenes (ABA), formed when two molecules of farnesylpyrophosphate combine to form squalene, a precursor of triterpenes (ursolic acid). Plants accumulate ABA when there is a water shortage. Mevalonic acid was possibly used to synthesize ABA, thereby inhibiting the production of ursolic acid.

Based on the research, it is clear that: (i) variations in water availability have a significant effect on the growth of *H. corymbosa* plants. The greater the availability of water, the greater the rate of growth. (ii) Variations in water availability did not affect ursolic acid compound levels in *H. corymbosa* plants. The highest level, 75.34 mg/g dry weight, was obtained at 100% KL water availability.

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