Yields and content of ursolic acid in pearl grass (Hedyotis corymbosa) when utilizing cow manure fertilizer in different shades

L D Jayanti¹, A Yunus², B Pujiasmanto² and Y Widyastuti³
¹Study Program of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia
²Study Program of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Indonesia
³Research center for Medicinal Plant and Traditional Drug, Tawangmangu, Indonesia

E-mail: yunus.uns7@yahoo.com

Abstract. This study aims to examine the intensity of shade and proper dosage of fertilizer to maximize the content of ursolic acid and pearl grass yield. The field-run study was carried out at the Medicinal Plant Garden of the Research and Development of Medicinal Plants and Traditional Medicines (B²P²TOOT) and the laboratory research was conducted at the B²P²TOOT Phytochemistry Laboratory. The experiment design was Randomized Complete Block Design with Split Plot pattern with the intensity (three levels) as main plot and fertilizer dosage (four levels) as sub plot. The data obtained were analyzed using the variance analysis, if there were significant differences tested further by using Duncan’s Multiple Range Test (DMRT) with 95% confidence level. The results showed that shade treatment gave significant effects on plant height, number of branches, root length, fresh weight and dry weight. The treatment of a fertilizer dosage 15 tons/ha gave the best results on fresh weight and dry weight. The combination of N₀K₀ treatment (without shade, without cow manure) resulted in the highest quality of ursolic acid as it featured a light blue color when detected under UV₃₆₆ light.

1. Introduction
Modern medicine recently is costly, as an alternative, many members of the community back to use trusted traditional medicine. Plants have been widely used by the community as a source of food and medicines for their generation [1]. One of the herbs that has been used as an herbal medicine and widely consumed by the community is pearl grass [1].

The pearl grass (Hedyotis corymbosa) is an herbaceous plant that almost all of the communities recognize it as weed or pest plants. Viewed in terms of the benefits this grass contains substances that has potential as an anti-cancer. Ursolate acid compounds which are contained by pearl grass are proved to prevent the development of cancer cell division to a more vicious stage [2], [3] states that whole pearl grass plants can be used as medicines. The efforts in improving the yield and quality of the pearl grasses are expected to take an important role to maintain the availability and maintain the
quality of the processed post-harvest. One effort that can be done is by applying cow manure as organic fertilizer and providing shade above the plantation. According to [4] organic farming is considered able to produce products that are quite safe and nutritious, so it can increase the positive effects in its use and once can increase the competitiveness of the agribusiness products. The use of the shade is expected to be able to know the increase of biomass of pearl grass harvest.

2. Methods

The research was conducted in July-November 2016 in Tanjungsari Village, Tegal Gede, Karanganyar. The analysis of secondary metabolite content was conducted at Indonesian Center for Research and Development of Traditional Medicinal and Medicinal Plants of Tawangmangu (B2P2TOOT). The tools used during planting are bucket, mortar, hoe, silver black mulch, gauge, knife or scissor, ruler, pegs, knapsack sprayer and hammer. The tools used to analyze secondary metabolites are TLC (Thin Layer Chromatography), pipettes, petri dishes, ovens, waterbaths, funnels, bottles, filter paper, sonicators and Chamber UV 366 and UV 256. The materials used for planting include pearl grass seed (Hedyotis corymbosa), moss soil media, sand, cow manure, paranet, bamboo and nail. Materials used for the analysis of sine metabolites are methanol (extraction agent), silica gel, ethyl acetate and formic acid.

This study uses Randomized Complete Block Design (RCBD) split plot pattern with 2 factors, they are cow manure and shade in three replicates. The shade that becomes the main plot in this study consists of 3 levels, they are control or shade 0% (N0), 50% shade (N1) and 75% shade (N2). Sub plot of this research is manure consisting of 4 levels which were control or without cow manure (K0), cow manure 10 tons/ha (K1), cow manure 15 tons/ha (K2) and cow manure 20 tons/ha (K3). The data obtained were analyzed using variance analysis (Anova) and if there were significant differences followed by DMRT with 95% confidence level. The observed variables are plant height, number of branches, root length, fresh weight of plant, dry weight of plants and secondary metabolite content of pearl grass.

3. Results and Discussion

3.1. Growth

3.1.1. Plant height Plant height is one variable that is often used to observe the growth process because the process of measuring plant height can be done in a simple way and does not disturb the plant. [5] and [6] argue that plant height is one of the most frequently observed plant sizes and is used as a parameter to measure the size of the effect of the applied treatments.

Table 1. The effect of shading on plant height

| Shade  | Plant Height (cm) |
|--------|-------------------|
| 0% (N0) | 28.80b            |
| 50% (N1) | 23.85a            |
| 75% (N2) | 24.04a            |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 1 shows the average height of the highest pearl grass obtained in the treatment without shade (N0) which is 28.80 cm. The average height of the lowest pearl grass obtained at the shade treatment of 50% (N1) is 23.85 cm. the provision of shading can reduce the acceptability of light by plants because plants receive the light that comes as in [6].

Based on the test results of the further given treatment, the dosage of cow manure has no significant effect on the height of pearl grass. It is suspected because the dosage given is still low and
has not been able to give the real effect to the height of the plant. The given fertilizer is organic fertilizer which has advantages and disadvantages as explained by [7], cow manure is an organic fertilizer that has less nutrient content than inorganic fertilizer or artificial fertilizer.

The growth of pearl grass at the age of 0-7 weeks after planting (WAP) is presented in Figure 1.

![Graph of pearl grass growth in 8 weeks.](image)

**Figure 1.** The graph of pearl grass growth in 8 weeks.

Figure 1 shows an increase of shade intensity from shade treatment without shade, 50% shade and 75% shade affect the average of pearl grass height per week. From this study it can be obtained the highest average pearl grass at the combination of N₀K₂ treatment (without shade and manure 15 tons/ha). Organic fertilizer has functions as a soil aggregate feeder and as an important source of soil and plant nutrients [5]. Basically pearl grass is a weed that can live in damp places and enough of light. Pearl grass does not demand on too much treatment, it can be done by simply watering in an adequate way, maintaining moisture and fertilizing the basic fertilizer and placed in a sufficient place for the sun [1].

### 3.1.2. Number of Branches

In environmental conditions with the sufficient light, photosynthesis process will be going well and produce high energy[8]. The higher the energy formed, the higher the number of plant organ size such as the number of branches and leaf area.

| Shade   | Number of Branches |
|---------|---------------------|
| 0% (N₀) | 13.3b               |
| 50% (N₁)| 5.4a                |
| 75% (N₂)| 6.5a                |

Table 2 shows the treatment without shade (N₀) yielding the highest average number of branches(13.3). The average number of the lowest branches was obtained in 50% shade treatment (N₁) of 5.4. [19] state that un-shaded plants will get high light intensity so the photosynthesis process will increase because light is a source of energy for photosynthesis process then this photosynthesis results will then be used to respond the shoot growth.

Based on the result of the analysis of variety effects of cow manure toward the number of branches, there is no significant difference was found. The treatment of cow manure at various levels does not have any effects on the observed variables, it might be due to the nutrient elements in the fertilizer are not enough for the formation of the new cells. It was explained that when plants get enough nutrients to actively form new cells, causing enlargement of plants toward the diameter of the stem and the formation of primary branches [10].
Figure 2. The graph of pearl grass increment in 8 weeks.

The treatment without shade with the provision of fertilizer dosage 15 tons/ha (N\textsubscript{0}K\textsubscript{2}) gives the highest number of branches with 15 branches. Plants increase body size in overall is the result of increasing the size of plant parts (organs); it is the effect of the increase of cell tissue produced by the increase of cell size [6].

The observed branches are the primary branches that can grow from various directions and cover the main stem. The branches on a plant form a certain angle with the main stem that cause the direction of growing branches are different [11]. In a shaded plant condition, plants also maximize their light capture by modifying the position of the leaves and the other organs to avoid a shade called avoiding response to shade [12].

3.1.3. Root Length Roots are part of the plant that has an important role in the growth of a plant.[13] explain that root is a part of the plant that has an important role in the adaptation of plants to water shortages because the roots are able to absorb water by maximizing the root system. In contrast with other grasses that have a fiber root system, [2] states that the root of the pearl grass is a tread root with a yarn-shaped root.

Table 3. The effect of shade level on the root length

| Shade     | Root Length (cm) |
|-----------|------------------|
| 0% (N\textsubscript{0}) | 12.2b            |
| 50% (N\textsubscript{1}) | 9.5a             |
| 75% (N\textsubscript{2}) | 9.4a             |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 3 shows the longest root length average obtained at the non-shaded treatment (N\textsubscript{0}) that is 12.2 cm long. The average of the lowest root length was obtained at the treatment of 75% (N\textsubscript{2}) shade which is 9.4 cm long. A shaded condition or a low light condition causes the auxin hormone works [14]. [8] explain that the auxin hormone will only stimulate the growth of new roots and does not cause a long increase on plant roots. In the [16] study the length and dryness data between roots and canopy in a shade treatment has a lower mean length and dry weight compared with controls. The result of the analysis of the variety of cow manure influence on the length of the pearl grass roots there is no significant difference in each treatment level. The possibility that can be predicted is the lack of fertilization dosage so the roots growth of pearl grasses does not make a significant difference.
3.1.4. Fresh Weight of Plant The fresh weight of the plant is a measure of the result of photosynthetic deposits in plants in the form of heavy plants that still contain water content which is not constant. Explains that plant weight can also show the result of plant metabolism activity and the wet weight is also influenced by the tissue water content.

**Table 4.** The effect of shade level on the fresh weight of plant

| Shade     | Fresh Weight (g) |
|-----------|------------------|
| 0% (N₀)  | 8.92b            |
| 50% (N₁) | 2.05a            |
| 75% (N₂) | 2.14a            |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 4 shows the highest average fresh weight obtained in the treatment without shade (N₀), it is 8.92 grams. The lowest average fresh weight was obtained at 50% shade treatment (N₁). The results are directly proportional to the average shade effect on plant height contained in Table 1. Fresh weight of pegagan in shade treatment gives less result compared with unregistered pegagan [17] while shaded arrowroots plants produce fewer end biomasses than un-shaded arrowroots plants due to less light availability to form assimilates [18].

The fertilizer used in this research was cow manure. Cow manure is one of organic fertilizers that is often used by farmers in general. The addition of organic materials into the soil such as fertilizer would have stronger influence towards the improvement of soil properties and not in particular to increase the nutrients in the soil [19].

**Table 5.** Effect of different dosages of cow manure on fresh plant weight.

| The Dosage of Fertilizer | Fresh Weight (g) |
|--------------------------|------------------|
| 0 (K₀)                   | 2.11a            |
| 10 tons/ha (K₁)          | 4.53ab           |
| 15 tons/ha (K₂)          | 5.86b            |
| 20 tons/ha (K₃)          | 4.97b            |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 5 shows the highest average fresh weight of pearl grass obtained in the treatment of manure 15 tons/ha it is 5.86 grams (K₂). The lowest average fresh weight of pearl grass was obtained in the treatment without fertilizer (K₀). This proves that the addition of cow manure or organic fertilizer can improve the yield of pearl grass. The study of [20] stated that organic fertilizers can give beneficial effects because it has the ability to improve soil quality.

3.1.5. Dry Weight of Plant Dry weight of plants is a measure used to know the results of photosynthesis of a plant with a constant water content. The dry matter of a plant is a assimilat reflection which is produced from the process of photosynthesis of the plant [8]. If the dried material produced by the plant is low then the assimilat produced by the plant is also low [20].
Table 6. The effect of shade level on the dry weight of plants

| Shade   | Dry Weight (g) |
|---------|----------------|
| 0% (N₀) | 4.40b          |
| 50% (N₁) | 1.46a          |
| 75% (N₂) | 1.64a          |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 6 shows the highest average dry weight obtained at the treatment without shade (N₀) of 4.40 grams. The lowest average dry weight was obtained at the 50% (N₁) treatment of 1.46 grams. The pearl grass grows well and produces high dry weight at full light intensity or treatment without shade (N₀). [21] who state that the dry weight of plants in the shade treatment will decrease because the intensity of light is reduced then followed by the rate of photosynthesis that will decrease as well.

The growth of a plant can decrease due to less optimum conditions, hence the input is needed to support the growth of the plant [22]. One of them is by doing fertilization. Fertilization is the most important factor in growth and development until the result of production [23]. He also states that fertilizing using organic fertilizer is as important as fertilizing by using inorganic fertilizer for plant growth process.

Table 7. The effect of different dosages of cow manure on the dry weight of plant

| The Dosage of Fertilizer | Dry Weight (g) |
|--------------------------|----------------|
| 0 (K₀)                   | 1.77a          |
| 10 tons/ha (K₁)          | 2.34a          |
| 15 tons/ha (K₂)          | 3.37b          |
| 15 tons/ha (K₃)          | 2.52ab         |

Description: The numbers followed by the same letter show no significant difference (P<0.05).

Table 7 shows the highest average dry weight obtained in the treatment of fertilizer dosage of 15 tons/ha (K₂). The lowest average dry weight was obtained in the treatment without fertilizer (K₀). Chemical fertilizers and organic fertilizers are equally important for plant growth, but it is recommended to use more organic fertilizer than chemical fertilizers [24]. In the study of [27] proves that the biomass of beans by using organic fertilizer is higher than the bean plant biomass by using urea fertilizer. Table 7. shows the increasing dry weight of no fertilizer (K₀), the fertilizer dosage of 10 tons/ha (K₁) to the fertilizer dosage of 15 tons/ha and decreased at fertilizer dosage 20 tons/ha (K₃). In accordance with Table 7, it can be concluded that the dosage of fertilizer that can maximize the dry weight of pearl grass in this study is the dosage of fertilizer 15 tons/ha (K₂).

3.2. Secondary Metabolite

3.2.1. Secondary Metabolite Content Analysis Plants produce secondary metabolite compound (secondary metabolite) [25] that can be used to cure chronic and lethal disease [26]. In the utility as a traditional medicine, pearl grass is formed into a dosage. It shows that pearl grass has a great potential to be developed into useful herbs and in more advanced production.
Based on the figure 3, it can be seen that the highest total extract level is obtained from the combination of treatment without shade and the dosage of manure 10 tons/ha (N₀K₁). The lowest extract level is obtained from the combination of treatment without shade and 15 tons/ha (N₀K₂) fertilizer dosage. This result is in contrast to the growth of pearl grass. The growth of the plant height and the number of pearl grass branches in the combination of treatment without shade and cattle manure 15 tons/ha (N₀K₂) yielded the highest result. Primary metabolite in plants is used by plants for growth [27]; whereas secondary metabolite is produced by plants do not directly have a role for the plant growth. The higher the total extracts level of pearl grass, the higher the secondary metabolite content contained in it.

3.2.2. Ursolate Acid Content Analysis One of the metabolites which is contained in pearl grass is ursolic acid. Pearl grass is a good source of oleanolate acid (OA) and ursolic acid (UA) and both compounds have many beneficial effects for human health [28]. This compound is an active anti-cancer compound that has long been used in the field of health. In Chinese health science, pearl grass has efficacy in the healing of fever, anti-inflammatory, diuretic, ulcers and also activate the blood circulation [28]. That statement is also supported by [29] which explain that ursolic acid has the potential to be developed as an anticancer compound due to its ability to inhibit several stages in carcinogenesis and supported by its widespread presence in many plants.
Ursolic acid is a chemical compound of triterpenoid pentacyclic group which is found in plants. The triterpenoid group belongs to terpenoid groups which are synthesized through the mevalonate pathway. Here are the results of Thin Layer Chromatography that has been done at the BP2TOOT Phytochemistry Laboratory.

The combination of treatment without shade and without fertilization (N₀K₀) produces a stronger color than other treatments. This can be expected because of the condition of pearl grass that experienced stress both light stress and nutrient stress so it produces side results to defend itself.

Various types of plants are able to produce secondary metabolite compounds in certain condition and secondary metabolite serves as a defense mechanism of plants, both from biotic and abiotic stress. On the other hand the combination of shade treatment of 50% and 20 tons/ha manure application (N₁K₃) produces a weak and almost faded violet color. The treatment produces the highest total extract content in the secondary metabolite content analysis. This proves that the pearl grass does not only contain ursolic acid and in this combination there is another stronger compound and it is not discussed in this research. The differences in the environmental conditions where the plants grow can also cause the differences of the amount of secondary metabolites contained in plants.

4. Conclusions
Based on the results of the study it can be concluded that, a treatment without shade (N₀) is expected to provide the highest ursolic acid quality in ursolic acid quality analysis by providing a bright blue spot color. A fertilization with dosage of cow manure 15 tons/ha (K₂) can produce the highest fresh weight with 5.86 grams and the highest dry weight 3.37 grams. A treatment without shade (N₀) and without cow manure (K₀) can give best quality of pearl grass appropriate with ursolic acid quality analysis.

Acknowledgement
This research was supported by the Medicinal Plant and Traditional Drug, Tawangmangu, Indonesia.

References
[1] Soemardji A S, Anisa I N, Damayanti N A 2015 Study on rumput mutiara (hedyotis corymbosa) herbs as medicine. J. Medicine and Health 1(2) 187-199
[2] Sirait N 2014 Potensi rumput mutiara (hedyotis corymbosa) sebagai antikanker Warta Penelitian dan Pengembangan Tanaman Industri 20(3) 11-13
[3] Endrini S 2011 Antioxidant activity and anticarcinogenic properties of “rumput mutiara” (hedyotis corymbosa (L.) Lam.) and “pohpohan” (pilea trinervia (Roxb.) Wight) J Med. Plant Res. 5(16) 3715-3718
[4] Roidah I Y 2013 Manfaat penggunaan pupuk organik untuk kesuburan tanah. J Universitas Tulungagung Bonoworo 1(1) 31-42
[5] Sonbai J H H, Prajitho D and Syukur A 2013 Pertumbuhan dan hasil jagung pada berbagai pemberian pupuk nitrogen di lahan kering regosol Ilmu Pertanian 16(1) 77-89
[6] Sitompul S M and Guritno B 1995 Analisis pertumbuhan tanaman Yogyakarta UGM Press
[7] Sompotan S 2013 Hasil tanaman sawi (brasicca junea L.) terhadap pemupukan organik dan anorganik Geosains 2(1) 14-17
[8] Suminarti N E 2016 Analisis pertumbuhan tanaman dan hasil ubi jalar (ipomoea batatas (L.) Lam.), Prosiding Seminar Nasional Pembangunan Pertanian
[9] Fauzi R, Meiriani and Barus A 2016 Pengaruh persentase naungan terhadap pertumbuhan bibit Mucuna bracteata D.C. asal setek dengan konsentrasi IAA yang berbeda J Agroteknologi 4(3) 2114-2126
[10] Zamriyetti and Rambe S 2006 Kajian pertumbuhan dan produksi varietas jagung lokal dan kacang hijau dalam sistem tumpangsari J Penelitian Bidang Ilmu Pertanian 4(2) 70-7
[11] Tjitrosoepomo G 1990 Morfologi tumbuhan Yogyakarta UGM Press
[12] Merelo P, Paredes E B, Heisler M G et al 2017 The shady side of leaf development: the role of the revoluta/kanadi1 module in leaf patterning and auxin-mediated growth promotion J. Curr. Opin. Plant Biol. 35 111-116

[13] Ai N S, Torey P 2013 Karakter morfologi akar sebagai indikator kekurangan air pada tanaman. J Biosilos 3(1) 31-39

[14] Buntoro B H, Rogomulyo R and Trisnowati S 2014 Pengaruh tekanan pupuk kandang dan intensitas cahaya terhadap pertumbuhan dan hasil temu putih (curcuma zedoaria L.) J Vegetalika 3(4) 29-39

[15] Husein E, Saraswati R and Hastuti RD 2008 Rizobakteri pemacu tumbuh tanaman. balittanah.litbang.pertanian.go.id. [5 Mei 2017]

[16] Khoiri M 2010 Pengaruh naungan terhadap pertumbuhan dan laju fotosintesis tanaman cabe merah (capsicum annuum L.) sebagai salah satu sumber belajar biologi. Download.portalgaruda.org. [30 April 2017]

[17] Tripatmasari M, Wasonowati C and Alianti VR 2010 Pemanfaatan naungan dan pupuk kotoran sapi terhadap pertumbuhan dan kandungan tripenoid pegagan (centella asiatica L.) Agrovivor 3(2) 137-145

[18] Begna S H, Dwyner L M, Cloutier D et al 2002 Decoupling of light intensity effects on the growth and development of C3 and C4 weed species trough sucrose supplementation J Exp. Bot. 53(376) 1935-1940

[19] Yang Y C and Wei M C 2016 A combined procedure of ultrasound-assisted and supercriticalcarbon dioxide for extraction and quantitation oleanolic and ursolicacids from Hedyotis corymbosa Ind Crops Prod. 79 7-17

[20] Ogbonna P E and Nweze N J 2012 Evaluation of growth and yield responses of cocoyam (colocasia esculenta) cultivars to rates of NPK 15:15:15 fertilizer. Afr. J Agric. Res. 7 6553-6561

[21] Gardner F P, Pearce R B, Mitchell R L 2005 Fisiologi tanaman budidaya Jakarta UI Press

[22] Musyarofah N, Susanto S, Aziz A A et al 2007 Respon tanaman pegagan (centella asiatica L. Urban) terhadap pemberian pupuk alami di bawah naungan Buletin Agronomi 35(3) 217-224

[23] Şener S 2016 Effects of several cultivars', mulch and fertilizer aplications on plant growth and development criteria and plant's nutrition elements uptake in organic strawberry plantation in Nevşehir city Asian Economic and Social Society 6(11) 221-228

[24] Liang Z, Jiang Z, Fong D W et al 2009 Determination of oleanolic acid and ursolic acid in Oldenlandia difussa and its substitute using high performance liquid chromatography. J. Food Drug Anal. 17(2) 69-77

[25] Haryanti S and Katno 2011 Aktivitas sitotoksik Ocimum sanctum L pada sel kanker kolon WiDr. Simposium Nasional XV PERHPBA Surakarta