The Ursolic Acid Content of Rumput Mutiara (Hedyotis corimbosa L.) Grown in Various Locations

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Abstract—Hedyotis corimbosa L. or Rumput Mutiara (Indonesian Local name) is one of the plants that is getting more famous as an herbal medicine and is consumed by the public to cure diseases or to maintain health. All parts of Hedyotis corimbosa L. can be used as an efficacious drug, which is an anti-inflammatory, anticancer, antipyretic, diuresis, blood circulation, antitoxin, etc. As an herbal ingredient, Hedyotis corimbosa L. is currently known as a wild plant that has not been popularly cultivated, thus the quality of Hedyotis corimbosa L. is varied. Therefore, it is necessary to map which areas produce Hedyotis corimbosa L. with highest ursolic acid. This study aimed to determine the content of ursolic acid in Hedyotis corimbosa L. from its various different growth locations. This research applied thin layer chromatography techniques and the analysis of variance with a level of $\alpha = 5\%$ using Unbalanced Incomplete Block Design. The results showed that site altitude treatment did not show any significant difference in the number of leaves, root length, plant height, node length, stem diameter, number of branches, leaf width, leaf length, flower stem length, total extract Yield, and ursolic acid levels. Still, it showed significantly different effects on water content, dry rendement, and productivity. The correlation analysis showed that there is no significant correlation at $\alpha = 5\%$ between ursolic acid content and root length, plant height, leaf width, leaf length, and total extract Yield while node length, stem diameter, number of branches, flower stem length, number of leaves, dry rendement and productivity showed a significantly positive correlation with ursolic acid content. Water content showed significantly negative correlation with ursolic acid content. The conclusion is that there is no significant difference in ursolic acid content from plants grown at low altitudes and medium altitude. However, the content of ursolic acid from medium altitude is relatively higher compared to ursolic acid content from low altitude. This implies that there is a tendency for ursolic acid content in Hedyotis corimbosa L. to be influenced by the conditions of its growth location so that cultivation treatment with different conditions will affect the ursolic acid content.

Keywords—secondary metabolites; chromatography; altitude; rumput mutiara; Hedyotis corimbosa L.

I. INTRODUCTION

One of the deadliest types of cancer is breast cancer [1]. Chemotherapeutic agents are the priority treatment for breast cancer cures. Among them, the mainly used agent is doxorubicin, which is a first-generation anthracycline antibiotic with a broad spectrum of chemotherapy actions [2]. Doxorubicin has several undesirable side effects, mainly related to cardiotoxicity. The use of doxorubicin risks causing side effects of cardiotoxicity and cancer cell resistance [1]. Efforts to cure this disease are generally still relatively expensive and have considerable side effects [3].

The analysis of ursolic acid single treatment on cell cycle showed inhibition in the G1 phase, while the single treatment of doxorubicin showed inhibition in the G2/M phase. The combination of ursolic acid and doxorubicin showed inhibition of the MCF-7 cell cycle in the G1 phase and shows indications of apoptotic induction. Thus, ursolic acid has the potential to be developed as a co-chemotherapy agent with doxorubicin [1]. Plants naturally produce various compounds which are classified into three categories, namely primary metabolites, polymers, and secondary metabolites [4]. Meanwhile, in general, there are two types of metabolism, namely primary metabolism and secondary metabolism [5].

Plants produce a variety of active compounds that provide pharmacological effects. Generally, these active compounds do not play an important role in plant metabolism, so they...
are often referred to as secondary metabolites [6], [7]. Secondary metabolites are also thought to be the waste or detoxification products of plants. However, most of the functions of secondary metabolites are still unknown [8], [9]. Research on secondary metabolites is still one of the largest research areas to determine the function and pharmacological properties of each secondary metabolite [9]. One of the medicinal plants that contain secondary metabolites, ursolic acid, is Hedyotis corimbosa L. with a potential as an anti-cancer drug [10], [11]. Hedyotis corimbosa L. has many secondary metabolites including ursolic acid and oleanolic acid [12]. Ursolic acid and oleanolic acid has the function of preventing the division of cancer cells to become more virulent [1]. The research of Hedyotis corimbosa L. resulted in the isolation of eight compounds, which were identified as ursolic acid, ferulic acid, 3β-hydroxyolean-11-en-28, 13β-olide, stigmastane-3,6-dione, β-sitosterol, 2-hydroxy-1-methoxyanthraquinone, 3-hydroxy-1, 2-dimethoxyanthra-quinone, scopeletin, [13]. Hedyotis corimbosa L. could cure ulcers, as antihepatotoxic, which can treat the liver from damage or inflammation and protect the liver and has the potential as anti-cancer [14].

Various studies have been conducted on the use of Hedyotis corimbosa L. in the fields of pharmaceutical and health including phagocytosis activity of mouse macrophages [15], toxicity tests [16], [17], cytotoxic activities [18], antioxidant activities [19], [18], antibacterial activity [20], anti-arthritis effect [21], total flavonoid test [22], and anti-carcinogenic effects [19], [23], [24], [25], as well as research on the content of secondary metabolites of Hedyotis corimbosa L. namely screening of secondary metabolites of Hedyotis corimbosa L. extract (Hedyotis corimbosa L.) with GEMS method [12], and oleanolic acid content and growth of Hedyotis corimbosa L. at various doses of cow manure and liquid organic fertilizer [26].

From the research that has been conducted, there is research on the inhibitor effect of Hedyotis corimbosa L. leave decoction on the growth of C3H mice breast tumors in vivo. The research result showed that the administrations of Hedyotis corimbosa L. leaf decoction at a dose of 0.25 mg/ml; 0.5 mg/ml; 1 mg/ml; and 2 mg/ml per day for 21 days orally inhibit the growth rate of breast tumors in C3H strain mice in vivo as reflected by tumor volume and AgNOR score. Further, the researcher recommends that as an improvement for future studies, it is necessary to consider using an increased dose of ursolic acid (secondary metabolites) [23].

Secondary metabolites are organic compounds produced by plants that do not have direct functions in photosynthesis, growth or respiration, solute transport, translocation, protein synthesis, nutrient assimilation, differentiation, the formation of carbohydrates, proteins, and lipids [27]. Primary metabolites play a role in the process of photosynthesis and respiration, while secondary metabolites play more roles in plant defense functions [5].

Secondary metabolites are produced through pathways outside the biosynthesis of carbohydrates and proteins [11], [28]. Primary metabolism is directly involved in growth, whereas secondary metabolism is not generally involved in growth activity [5].

The research explained that environmental conditions affect productivity, especially levels of ursolic acid (secondary metabolites) [28]. The higher the altitude is, the higher the environmental stress gets, for example, the lower the temperature is, the higher the humidity, the smaller the intensity of sunlight, and the shorter the exposure time gets. Temperature stress, light, humidity, and others can affect the production of plant secondary metabolites [29]. The research explained that different chemical compositions would inevitably lead to different biological effects of Hedyotis corimbosa L. in clinical application [30].

Hedyotis corimbosa L. contains ursolic acid, gamma sitosterol, and oleanolic acid. It was found that the plant contains up to 0.12% of biflorin (White crystalline powder) and alkaloids-bifloron (yellow crystalline powder) [31]. This study aimed to determine the ursolic acid content of Hedyotis corimbosa L. from various growth locations.

II. MATERIAL AND METHOD

The study was conducted by field experimental method with two treatments include low and medium altitude in 21 different locations, which arrange Incomplete Block Design with 15 and 6 locations, respectively, as replications. This research was carried out in December 2019, at the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Tawangmangu.

A. Materials

Materials include Hedyotis corimbosa L. simplicia, methanol (MeOH), ursolic acid standard, filter paper, hexan, toluent, ethyl acetate, glacial acetic acid, silicone grease, methanol (MeOH), ursolic acid standard, filter paper, hexan, toluent, ethyl acetate, glacial acetic acid, silicone grease, analytical chromatography and spotting solution (Dragendorf reagent). Tools include rulers, knives, oven, blenders, digital scales, simplicia containers, boilers, pipettes, measuring cups, test tubes, test tube caps, funnels, spotters, sprays, sprayers, Thin Layer Chromatography (TLC) visualizer and computers. The method applied in this research was Thin Layer Chromatography.

The collection of Hedyotis corimbosa L. material was conducted with the criteria that have entered the generative phase in 21 different locations of the growth place (Figure 1). On the map, the location shows that the red dot is the location of Rumput Mutiara sampling, which is spread from low altitude and medium altitude in Central Java. There were 21 sampling locations. Sampling was done after knowing there were some Rumput Mutiara that grew in these locations; the location of the sampling was limited to the distance of 25 m². Plants taken have entered the generative phase.

B. Research Implementation

1) Preparation of raw materials as a simplicial: Wind drying Hedyotis corimbosa L. without sunlight. Chopping Hedyotis corimbosa L. into small pieces. Baking Hedyotis corimbosa L. in temperatures of ± 40 °C for 24 hours until the water content is gone. Mashing Hedyotis corimbosa L. until it was smooth and became simplicia powder that was ready to be extracted.
2) Extraction: This step weighed 100 mg of *Hedyotis corimbosa* L. simplicia powder. Also, this step dissolved 100 mg *Hedyotis corimbosa* L. simplicia powder in methanol (MeOH) 100 ml and made it into 100 mg/100 ml simplicia solution. Extraction also prepared ursolic acid standard, which was 1 mg of ursolic acid dissolved in 1 ml of methanol (1 mg/1 ml = 1000 µg/ml). Then, boiled simplicia solution and standard solution at 30 °C for 15 minutes. After the solution cooled, filtering was conducted.

3) Analysis of ursolic acid content: some steps conducted in this part. Firstly, preparing the toluent driving phase, ethyl acetate, glacial acetic acid (70: 30:2) less than 1 cm thick in the Thin Layer Chromatography (TLC) chamber. Secondly, performing a drive phase saturation check. Thirdly, preparing analytical chromatography paper with a size of 10 x 20 cm. Fourthly, bottling all simplicia every 10 µg/ml and ursolic acid standard that is 0.1 µg/ml, 0.2 µg/ml, 0.3 µg/ml, 0.4 µg/ml, 0.5 µg/ml on the TLC analytical chromatography plate using a sprinkler (linomat) with a distance of 1 cm from the bottom of the paper. Fifthly, inserting the Analytical Chromatography paper that had been marked in from the bottom of the paper. Sixthly, making observations on Analytical Chromatography paper that had been marked in from the bottom of the paper. Fifthly, inserting the plate using a sprinkler (linomat) with a distance of 1 cm

one and the other looks different, this shows that the content of ursolic acid is not the same between locations. The higher the versa, the low line means the ursolic acid content is also low. a prominent line means the high ursolic acid content and vice

### Table I

| Code | Location | UAC | masl | Latitude  | Longitude |
|------|----------|-----|------|-----------|-----------|
| L1   | Kendus   | 0.09| 59.8 | -6.4645   | 110° 5010'|
| L2   | Tembalang| 0.21| 212.0| -7.316    | 110° 2639'|
| L3   | Kepu     | 0.17| 72.6 | -7.80     | 111° 3538'|
| L4   | Marina   | 0.14| 2.0  | -6.5710   | 110° 2329'|
| L5   | Gajah Mwakur| 0.19| 103.0| -7.023    | 110° 2452'|
| L6   | Laweyan  | 0.16| 128.8| -7.3311   | 110° 4726'|
| L7   | Sidoharjo| 0.16| 200.0| -7.497    | 111° 333   |
| L8   | Godong   | 0.20| 19.2 | -7.223    | 110° 4058'|
| L9   | Banyumanik| 0.22| 374.6| -7.716    | 110° 2417'|
| L10  | Girimarto| 0.20| 448.3| -7.4730   | 111° 429   |
| L11  | Temanggung| 0.19| 448.9| -7.2256   | 110° 1348'|
| L12  | Rembang  | 0.18| 57.4 | -6.4215   | 111° 2045'|
| L13  | Pati     | 0.19| 41.8 | -6.4424   | 111° 146   |
| L14  | Kendal   | 0.15| 33.3 | -6.5747   | 110° 855   |
| L15  | Pekalongan| 0.22| 62.8 | -6.5410   | 109° 4119'|
| L16  | Batang   | 0.19| 160.5| -6.5812   | 109° 5210'|
| L17  | Jepara   | 0.15| 23.1 | -6.3527   | 110° 3857'|
| L18  | Baturaden| 0.20| 452.0| -7.1931   | 109° 1342'|
| L19  | Muntilan | 0.16| 357.3| -7.3429   | 110° 1622'|
| L20  | Slemen   | 0.16| 170.7| -7.4554   | 110° 3142'|
| L21  | Pemalang | 0.15| 28.1 | -6.5258   | 109° 2246'|

**Note:** masl: m above sea level

**Fig. 2** Chromatogram profile of *Hedyotis corimbosa* L. methanol extract originating from various regions. 

**Table 1** Data of *Hedyotis corimbosa* L. Observation and Analysis of Ursolic Acid Content 

- **Code**: L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14, L15, L16, L17, L18, L19, L20, L21: Medium Altitude
- **L**: 2, 9, 10, 11, 18, 19: Low Altitude
- **L**: 1, 3, 4, 5, 6, 7, 12, 13, 14, 15, 16, 17, 20, 21: Medium Altitude

**III. RESULTS AND DISCUSSION**

The Location, Altitude, Latitude, Longitude, and Code of *Hedyotis corimbosa* L. were obtained in Table 1. From the observations on *Hedyotis corimbosa* L. and analysis of TLC densitometry, the following results were obtained in Figure 2. Figure 2 showed that the appearance of the chromatogram profile of *Hedyotis corimbosa* L. methanol extract originating from various regions on TLC observation of 366 times appears red spot. After analytical chromatography and spotting solution (Dragendorf reagent), ursolic acid spot is visible with pink (366 spray lb). Figure of ursolic acid after analytical chromatography and spotting solution from *Hedyotis corimbosa* L. with various locations is presented in Figure 3. Figure 3 showed that the content of ursolic acid is shown on a prominent line. The height of the line between
Fig. 3 Ursolic acid from *Hedyotis corimbosa* L. with various locations

The regression analysis of the altitude to the dry rendement *Hedyotis corimbosa* L. were obtained in Figure 4. The relationship between altitude and dry rendement followed the power pattern significantly with the equation $Y = 0.615 + 0.109 \ln X$, with a probability error of $<0.009$. (Figure 4).

![Dry Rendement](image)

Fig. 4 Power Regression of Altitude and Dry Rendement

The response of the *Hedyotis corimbosa* L. plant to differences in altitude are presented in Tables 2 to Table 6.

**A. Plant Height, Leaf Number, and Root Length**

Plant Height, Leaf Number, and Root Length of *Hedyotis corimbosa* L. were obtained in Table 2.

| Treatment | Plant Height (cm) | Leaf Number | Root Length (cm) |
|-----------|------------------|-------------|------------------|
| Low       | 32.73 ± 5.57 a   | 382.20 ± 47.72 a | 8.57 ± 2.39 a    |
| Medium    | 34.00 ± 8.58 a   | 407.50 ± 53.04 a | 8.15 ± 2.28 a    |
| Average   | 33.10 ± 6.36     | 389.43 ± 49.34  | 8.45 ± 2.31      |

The average number followed by the same letter in a column was not significantly different in F Test at $\alpha = 5\%$. Table 2 showed that there was no significant difference in the height of plants from low altitude and medium altitude.

**B. Node Length, Stem Diameter and Branch Number**

Node Length, Stem Diameter, and Branch Number of *Hedyotis corimbosa* L. were obtained in Table 3.

| Treatment | Node Length (cm) | Stem Diameter | Branch Number |
|-----------|------------------|---------------|---------------|
| Low       | 2.14 ± 0.24 a    | 0.11 ± 0.02 a  | 12.20 ± 3.22 a |
| Medium    | 2.12 ± 0.34 a    | 0.11 ± 0.01 a  | 13.17 ± 2.71 a |
| Average   | 2.13 ± 0.27      | 0.11 ± 0.01    | 12.48 ± 3.12   |

The average number followed by the same letter in a column was not significantly different in F Test at $\alpha = 5\%$. Table 3 showed that there was no significant difference in the length of segments from low and medium altitudes, the diameter of the stem from the low altitude and medium-altitude, and the number of branches from medium altitude and low altitude.

**C. Leaf Width, Leaf Length, and Flower Stem Length**

The leaf width, leaf length, and flower stem length of *Hedyotis corimbosa* L. were obtained in Table 4.

| Treatment | Leaf Width (cm) | Leaf Length (cm) | Flower Stem Length (cm) |
|-----------|----------------|-----------------|-------------------------|
| Low       | 0.52 ± 0.16 a  | 2.03 ± 0.27 a   | 0.71 ± 0.15 a           |
| Medium    | 0.62 ± 0.17 a  | 2.00 ± 0.27 a   | 0.70 ± 0.09 a           |
| Average   | 0.55 ± 0.17    | 2.02 ± 0.26     | 0.71 ± 0.14             |

The average number followed by the same letter in a column was not significantly different in F Test at $\alpha = 5\%$. Table 4 showed that there was no significant difference in the leaf width from a low altitude and medium-altitude, leaf length from low altitude and medium-altitude, and length of the flower stems from low altitude and medium altitude.

**D. Water Content, Dry rendement, and Productivity**

The water content, dry rendement and productivity of *Hedyotis corimbosa* L. were obtained in Table 5.

| Treatment | Water Content (%) | Dry Rendement (g) | Productivity (g) |
|-----------|-------------------|-------------------|------------------|
| Low       | 74.18 ± 6.42 a    | 0.26 ± 0.06 b     | 0.04 ± 0.02 b    |
| Medium    | 66.82 ± 5.68 b    | 0.33 ± 0.06 a     | 0.07 ± 0.02 a    |
| Average   | 72.08 ± 6.97      | 0.28 ± 0.07       | 0.05 ± 0.02      |

The average number followed by the same letter in a column was not significantly different in F Test at $\alpha = 5\%$. Table 5 showed that there was a significant difference in the water content from low altitude and medium-altitude; the water content from medium altitude was significantly lower compared to the water content from low altitude. There was
a significant difference in the dry rendement from low altitude and medium-altitude, and the dry rendement from medium altitude was significantly higher compared to the dry rendement from low altitude. There was a significant difference in productivity from low altitude and medium altitude and that the productivity from medium altitude was significantly higher compared to productivity from low altitude.

E. Total Extract Yield and Ursolic Acid Content

The water content, dry rendement, and productivity of *Hedyotis corimbosa* L. were obtained in Table 6.

| Treatment | Total Extract Yield (%) | Frolic Acid Content (%) |
|-----------|--------------------------|-------------------------|
| Low       | 7.34 ± 1.81 a            | 0.17 ± 0.03 a           |
| Medium    | 7.30 ± 1.65 a            | 0.20 ± 0.02 a           |
| Average   | 7.33 ± 1.82             | 0.18 ± 0.02             |

The average number followed by the same letter in a column was not significantly different in F Test at α = 5%.

Table 6 showed that there was no significant difference in the total extract Yield from low altitude and medium-altitude, and ursolic acid content from low altitude and medium altitude. The analysis of variance with Unbalanced Incomplete Block Design showed that the treatment of the altitude did not show any significant difference in the number of leaves, root length, plant height, segment length, stem diameter, number of branches, leaf width, leaf length, flower stalk length, total extract Yield, and ursolic acid content. Still, it showed a significant difference in water content, dry rendement, and productivity. The altitude of the place affects the water content, dry rendement, and productivity.

F. Correlation the all parameter of *Hedyotis corimbosa* L.

The results of the study were analyzed for correlation, so the following findings were obtained in Table 7. The results of the correlation analysis showed that root length, plant height, leaf width, leaf length, and total extract Yield did not have a significant correlation at α = 5% on ursolic acid content. In contrast, node length, stem diameter, number of branches, length of the flower stem, number of leaves, dry rendement, and productivity showed a significantly positive correlation with ursolic acid content. Water content showed a significantly negative correlation with ursolic acid content. The regression analysis of the water content, dry rendement, dan productivity to the Ursolic Acid Content were obtained in Figures 5, 6, and 7.

The relationship between water content and ursolic acid content followed the cubic pattern significantly with the equation $Y = 0.247-56,547X+1,555E-5X^3$, with a probability error of <0.001 with $R^2$ value 0.838 (Figure 5).
The relationship between dry rendement and Ursolic Acid Content followed the logarithmic pattern significantly with the equation \( Y = 0.316 + 0.108\ln X \), with a probability error of <0.001 with \( R^2 \) value 0.842 (Figure 6).

The relationship between productivity and ursolic acid content followed the cubic pattern significantly with the equation \( Y = 0.013+7.118X-105.606X^2+600.966X^3 \), with a probability error of <0.001 with \( R^2 \) value 0.958 (Figure 7).

According to the results and discussion, the following conclusions can be drawn that findings showed that the growth location altitudes showed significant differences in water content, dry rendement, and productivity. The growth location altitudes did not show any significant differences in the number of leaves, root length, plant height, node length, stem diameter, number of branches, leaf width, leaf length, flower stem length, total extract Yield, and ursolic acid content. However, the content of ursolic acid from medium altitude is relatively higher compared to ursolic acid content from low altitude. This implies that there is a tendency for ursolic acid content in Hedyotis corimbosa L. to be influenced by the conditions of its growth location so that cultivation treatment with different conditions will affect the ursolic acid content.

Correlation analysis results show that root length, plant height, leaf width, leaf length, and total extract. Yield did not have a significant correlation at \( \alpha = 5\% \) on ursolic acid content. In contrast, node length, stem diameter, number of branches, flower stem length, number of leaves, dry rendement, and productivity show a significantly positive correlation with ursolic acid content. Water content showed a significantly negative correlation with ursolic acid content.

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