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Analyses of Bioactive Compounds of Pegagan (Centella Asiatica (L.) Urb) from Samosir – Indonesia Accession

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Abstract. *Centella Asiatica* or Pegagan is classified as one of the wild plants that has not been domesticated. The excessive usage of this plant in traditional and modern medicinal applications threatens its population and sustainability. Thus, to preserve the plant and supply the high request of this plant in agromedicinal industry, studies concerned with the growth and bioactive compounds of Pegagan cultivated under commercial field conditions are urgently needed. This study purposed to examine the bioactive components of Pegagan (especially in leaves and roots) under field conditions, including asiaticoside, madeccasoside, and Asiatic acid. The Pegagan was harvested weeks after planting (WAP). The wet and dry weights of the leaves and roots were weighted and subsequently measured for their centelloside compounds by Thin-Layer Chromatography (TLC) procedure. The results revealed that the resulting asiaticoside content in the roots (1.25%) was higher than in the leaves (0.88%). The same results were achieved for the madecassoside content where the madecasosside content in the roots was 2.23%, while the content in the leaves was 2.11%. However, contrarily, the Asiatic acid compound in the leaves was 1.10% higher than the content in the roots (0.60%). It might be attributed to a longer period of field cultivation of Pegagan that delivered adequate time for the plant to alter Asiatic acid to asiaticoside and madecasosside at a later developmental growth. Moreover, these discoveries are advantageous in defining the most proper harvest time for commercial field cultivation of Pegagan to yield the highest amount of certain centelloside compounds.

Keywords: asiaticoside, asiatic acid, madeccasoside

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1. Introduction

The term ‘Back to nature’ is one of the main goals in the development of a modern human culture where traditional herbal plants are regaining their popularity as alternative medicinal compounds. This development was first observed in developed countries three decades ago and has had a significant impact recently in developing countries as a major source and knowledge of nanotechnology and medicinal plants [1]-[3]. Lately, Japan has been the main importer of medicinal and aromatic plants from China and India which these two countries dominate the international supply of these plants [4]. One of the well-known marketed and extensively

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consumed medicinal plants is Centella Asiatica or frequently recognized as Indian pennywort or Pegagan [2], [5] or Pegagan in Indonesia [6].

Pegagan is still classified as a wild plant [7], and its domestication efforts are currently underway [8]. Besides its threatened status due to excessive exploitation [9], the benefits of Pegagan constitute the importance of this plant species to be domesticated [10]. Pegagan contains several known bioactive compounds such as saponin, including asiaticoside [11]. Asiaticoside has the function of quickening the wound healing process and helps treat diseases of leprosy and tuberculosis [12]-[14]. Saponins function to inhibit the excessive production of scar tissue (or called keloids inhibitor) [13]. In addition, Pegagan is also known or believed for its various benefits from improving blood cleansing and circulation, fever remedy (antipyretic) to treating neural memory degeneration, bacterial infection, tonic, muscle spasm, inflammatory and allergy [15], [16].

Pegagan can reach up 100 tons per month, where PT. Sidomuncul, one of the largest agromedicinal corporations in Indonesia, needs at least two to three tons/month (Hikmat et al, 2011). The supply of Pegagan from the wild population can only meet the national demands up to a maximum of four tons per month. Limited commercial cultivation of Pegagan only provides a fraction of the market demands and is still hampered by problems of inconsistent quantity and bioactive compounds quality. Therefore, large-scale cultivation and maintaining the uniform quality of the plant's bioactive compounds are the main priority [18], [19].

Studies regarding the content of secondary metabolites in Pegagan were conducted mostly on laboratory scale involving in vitro or whole plant cultures, such as those conducted by Alqahtani [7], Kim [20], Lambert et al. [21] and Mangas [22]. There were few or non-existent studies focused on determining the level of secondary metabolites of Pegagan grown under a field condition that represents a viable production system and commercially profitable Pegagan cultivation. One of the few studies was conducted by Vinolina and Siregar [23], who revealed that different Pegagan accessions have different levels of asiaticoside compound. They found that Samosir accession has the highest concentration of asiaticoside (2.38 %), followed by Kabanjahe (1.43 %), Medan (1.38 %), Berastagi (1.38%), and shaded-Samosir accession (0.28%). However, they used wild accessions of Pegagan, which might have grown in different environmental conditions than what is expected in commercial cultivation fields. The contrasting findings of Kim [20] and Mangas [22] regarding the asiaticoside components in the leaves and roots of Pegagan cultivated under laboratory condition also needs to be reconfirmed through different cultivation conditions such as field condition. Therefore, this recent study purposed to determe the number of centellosides (asiaticoside, madecasosside, and asiatic acid) in different parts of Pegagan cultivated under field conditions and low altitude. It is expected that the result of this study will provide new insight regarding the potential cultivation of Pegagan in low-lying areas and its expected content of highly beneficial centellosides.

2. Materials and Methods

2.1. Cultivation Field and Research Preparation

The cultivation field was placed in the experimental area of Pasar Satu Street, Medan, North Sumatra. It is located at 30 m height above sea level. This study lasted for four months, from June to September 2019. The pH, organic carbon (C), nitrogen, available phosphor, and K_2O of the soil at the cultivation field were measured via potentiometric, Walkley-Black volumetric, Kjedhal volumetric, spectro-volumetric, and atomic absorption spectroscopic (AAS, HCL 25%) procedures, respectively. All soil samples were examined at the Indonesian Oil Palm Research Institute laboratory, North Sumatra, Indonesia. Afterward, weeds were removed from the filed. Soil cultivation was done to build 10 units of soil beds sized 30 cm, or 1 m x 1 m- sized plots. To facilitate access between plots and retain the plants' separation in different plots, the plots was separated by distance of 0.5 m. The soil liming employed dolomite (150 g/plot) one week before the seed planting to increase the pH which was from pH 5.5 to 6.0.

2.2. Seed Planting and Maintenance

Pegagan seed used in this study was from the accession of Samosir, North Sumatra, Indonesia. The accession was selected because Samosir accession grows well in a field and needs to be tested for its growth and centellocide content. The mother plants were cultivated for two and a half months in plastic bags until they produced a single stolon which was used as the seed. The gathered stolons were imbedded immediately after being detached from the mother plants. Each plot was planted with four seeds where each seed was distanced by 40 cm. Soil fertilization used KCL and Urea and was finished three times at 0, 20, and 40 days after planting (DAP) during the cultivation period. The doses used during fertilization period were 22 g/plot for KCL and 30 g/plot for Urea. The fertilizers were spread uniformly over the planting holes.

Maintenance on the plants' cultivation was performed by employing consistent watering, weeding and plants replanting. Watering was continuously completed every afternoon by seeing the weather situations in the field. Weeding was finished daily by detaching weeds from the soil manually. Embroidery was completed two weeks after planting to change dead plants. Pests and disease control were conducted every week to avoid or block their distribution. The plant harvesting was conducted at 12 weeks after planting (WAP) by gathering all plant parts.

2.3. Measurement of Growth Characteristics

The leaves number was counted weekly during the 12 WAP period. The counted leaves were fully formed and open leaves, while yellowish dry leaves were not counted. The number and length of the primary tendrils and secondary tendrils were also computed every week during the 12 WAP period. The primary tendrils include tendrils emerging from the main plants, while the secondary tendrils are tendrils from the primary ones. The stolons formed were stolons coming out from the tendrils, which was also estimated weekly during the 12 WAP period.

2.4. Measurement of Wet and Dry Weight

Harvested Pegagan plants were divided by: the leaves samples comprising the leaves and petiols, and the root samples containing the roots, tendrils, and stolons. The samples were weighed for their wet weight. Plant biomass was measured at the harvesting time that was at the 12th WAP. The harvested plants were divided into the shoot and root and dried using an oven at 50°C for 72 hours.

2.5. Analysis of Centellosides Content

The contents of centellosides in different parts of the harvested Pegagan were analyzed at Research Institute for Spices and Medicinal Plants, Bogor. The contents of different centelloside compounds (asiaticoside, madecassoside and asiatic acid) in Pegagan, especially in the leaves (L) and the roots (R), were determined using TLC process. The accumulation of the three centellosides compounds in above ground (leaves and petiole) and underground organs (roots and tendrils) were analyzed at harvest. The samples were then ground to form smooth powder to be used in the centelloside compound analyses. The centelloside was determined using CAMAG® TLC Scanner version 3, Switzerland. The wavelength used for asiaticoside, madecassoside, and asiatic analysis were 276 nm, 310 nm, and 290 nm, respectively [24].

3. Results and discussion

The bioactive substances in the leaves and roots of Pegagan uder commercial cultivation field with a harvest time of 12 WAP and analyzed using TLC are presented in Figure 1.

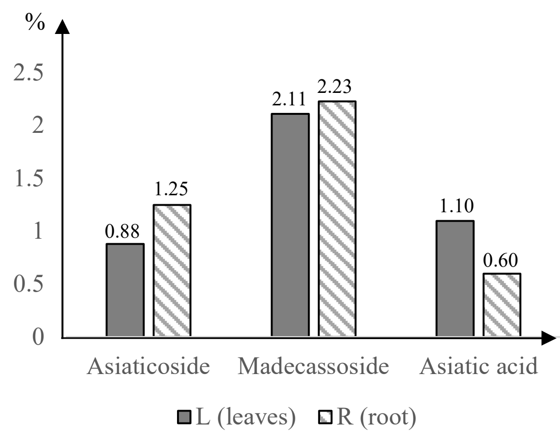

Figure 1. The content of Centelloside Compounds in the Leaves (L) and root (R) of Pegagan at 12 WAP

According to Figure 1, the asiaticoside and madecassoside contents of harvested Pegagan were found higher in the roots (1.25% and 2.23%) compared to the leaves (0.88% and 2.11%). Contrarily, the asiatic acid contents were higher in the leaves (1.10%) than in the roots (0.60%).

The measurement of growth characteristics of cultivated Pegagan during the 12 WAP is provided in Table 1. There was a sudden increase of growth at 6 WAP across all measured growth characteristics, of which the length of primary tendrils had the highest increase, up to 3 times compared to the average week by week tendrils length or other growth characteristics (leaves and number of primary tendrils, secondary tendrils, and stolons). This increase in tendrils length at 2 WAP was not considered a sudden increase despite the tendrils having a growth increase of more than three times from 1 WAP. All in all, the growth parameters showed generally normal development of the Pegagan plant.

Table 1. The Average Number of Leaves, Tendrils, and Stolon of the Cultivated Pegagan Observed Every Week During the 12 WAP Period

Parameter	Week after planting (WAP)											
	1	2	3	4	5	6	7	8	9	10	11	12
Number of leaves of the mother plant	3.0	5.4	7.2	9.2	11.8	14.0	15.0	19.0	20.4	23.4	24.4	26.2
Number of primary tendrils (tendrils coming out of parent)	0.6	1.0	1.2	2.4	3.2	4.6	7.0	7.6	9.8	10.4	11.2	12.0
Length of primary tendrils (cm)	1.6	7.6	12.0	16.2	30.6	88.4	146	207	287.4	365	440.2	518.6
Number of secondary tendrils (tendrils coming out of primary)	0.0	0.2	0.4	1.2	1.2	8.4	7.2	8.8	11.8	12.8	13.4	13.8
Number of Stolon	0.0	1.2	2.8	6.0	12.0	19.8	34.6	51.6	73.4	97.0	119.4	141.4

The comparison of harvested Pegagan's wet and dry weights for leaves, roots, and the whole plants averaged from the cultivation plots is provided in Table 2. The wet dan dry weights of leaves, roots and the whole plants of harvested Pegagan showed similar consistency where the whole plant has the highest ratio of the wet dan dry weights. This indicates that some parts of the harvested Pegagan were not categorized as roots and leaves and were discarded during the measurement of wet and dry weights.

Table 2. The Average and Ratio of Wet and Dry Weights of Leaves, Roots, and Whole Plant of Harvested Pegagan After 12 WAP

Parameter	Average (g)	Ratio of wet and dry weight
Wet weight of leaves	280.926	7.5
Dry weight of leaves	37.46	
Wet weight of roots	318.114	7.7
Dry weight of roots	41.38	

Table 3 shows the chemical composition of the soil beds before (#1) and at 0 WAP (#2). As stated in the methods section, the soil liming has successfully increased the soil bed's pH from 5.0 to 6.0. The content of phosphor and K20 were also doubled as a result of the fertilizer application.

Table 3. Soil Chemical Properties Measured Before Soil Cultivation and at 0 WAP

Soil Sample Code	Based on dry weight (heated at 105 ^{0}C)					
	pH	C (%)	N (%)	C/N	P (ppm)	K_2O
#1	5.0	5.77	0.58	10	6.67	0.01
#2	6.0	5.42	0.54	10	14.56	0.02

In this study, the leaves and roots of Pegagan from Samosir Accession were found to possess different concentrations of centelloside compounds. The higher concentration of asiaticoside and madecasosside in the roots found in this study was interesting and similar to that of the findings of Mangas et al. [22], where the asiaticoside and madecasosside contents in the roots were higher than in the leaves. This, however, contradicts the findings of other researchers such as Kim et al. [20], who had shown that the leaves contained more asiaticoside and madecasosside compared to the roots. Mangas et al. [22] explained the different results from Kim et al. [20] were due to a faster exposure of elicitors on the roof compared to that of the leaves. This phenomenon has caused a faster formation of the secondary metabolites (i.e. centelloside) in the roots. This study, however, did not use elicitors. Thus, we argue here that the longer cultivation period used in this study (12 weeks or 84 days) had allowed the transformation of asiatic acid in the cultivated Pegagan to asiaticoside and madecasosside in the roots. This research result was in line with the findings of Vinolina et al. [23], who found that the contents of asiatic acid and asiaticoside-madecasosside in Pegagan's leaves and roots showed an opposite trend over a culture period. They found that the asiatic acid content was higher while the asiaticoside-madecasosside contents were lower during the early cultivation period. The content of the former was, later on, decreased followed by the increase of the latter as the plant aged toward harvest time [18]. In addition, the sudden and significant rise in growth characteristics measured at 6 WAP was argued as the result of the third fertilization at day 40 or two days before the growth measurement at 6 WAP.

This research assessed the contents of centelloside compounds in Pegagan cultivation as initial data. This study showed the content of centellosida in Pegagan with a harvest time of 12 WAP with no treatment given to Pegagan from accession Samosir (highland accession) planted in the lowlands. In future research, the harvest times that are more than 12 WAP can be conducted on the leaves and roots to observe differences in the content of centelloside at a longer growth period of cultivated Pegagan so that the dynamics of centelloside biosynthesis can be understood.

4. Conclusion

Centella asiatica, Samosir accession has good growth in the lowlands. This study found that the asiaticoside content in the roots (1.25%) of of the harvested plants was higher than in the leaves (0.88%). Similarly, the madecasosside content in the roots (2.23%) was also higher compared to the content in the leaves (2.11%). Contrarily, the asiatic acid content in the leaves (1.10%) was higher than the content in the roots (0.60%). Moreover, this study discovered that the longer cultivation period in the field caused more alteration of asiatic acid into asiaticoside and madecassoside in the Pegagan plants.

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