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# Research Article
# Effect of Harvest Time on Bioactive Compounds of Field-cultivated *Centella asiatica* (L) Urban

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## Abstract

**Background and Objectives:** Pegagan, *Centella asiatica* (L) Urban is an important traditional herb and its wild population is currently heavily exploited due to commercial cultivation shortage. This study evaluated the content of different bioactive compounds in the leaves and roots of Pegagan, *Centella asiatica* (L) Urban cultivated in field condition and harvested at different times. **Materials and Methods:** The contents of centellosides in the leaves and roots of field-cultivated Deli Serdang Pegagan Accession were determined by using ultra fast liquid chromatography system. The levels of bioactive compounds in relation to harvest time were compared with an additional discussion on the dry/wet weight relationship. **Results:** The result showed that asiaticoside and madecassoside increased while asiatic acid decreased over time in both the leaves and roots of Pegagan. However, the leaves were shown to have significantly higher amounts of all centellosides compared to the roots in all harvest time treatments. **Conclusion:** This study concluded that a late harvest of Pegagan is best to maximize the production of asiaticoside and madecassoside. Conversely, early harvest was best to the maximum amount of asiatic acid in Pegagan.

**Key words:** *Centella asiatica*, bioactive compounds, asiaticoside, chromatography system, centellosides

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Centella asiatica* is widely known as Gotu Kola in the international market or 'Pegagan' in Indonesia[1]. It is known for its bioactive compounds and has been used as a medicinal ingredient to treat various illnesses and diseases[2-4]. Such benefits offered by this tropical plant have led to its commercial cultivation in some of the dominant producing countries such as China and India[5,6]. However, the commercial cultivation of Pegagan in Indonesia is lagged behind due to the inconsistent supply of quantity and quality due to environmental conditions, harvest and post-processing[7]. As a result, the increasing national and international demands of Pegagan is primarily supplied from the wild population[8] resulting in overexploitation of the plant worldwide[9].

Previous studies on the content of bioactive compounds in Pegagan such as asiaticoside, madecassoside and asiatic acid have been focusing on their complex and benefits of biochemical processes[10], their concentration in different parts of the plant[11-13] and general reviews of its growth under controlled conditions[11,14]. There was limited or non-existent literature or studies, particularly within Indonesian regions, that provide information regarding the effect of harvest time on the concentration of important bioactive compounds of Pegagan cultivated in field condition. Such discrepancy has been highlighted by Vinolina *et al.*[7] and Ghulamahdi *et al.*[15] where the field production of Pegagan varies in terms of quality and quantity throughout the year. Field location, seasonal climate condition as well as accession type contributed to these inconsistencies are needed to be studied further[10,16]. Hence this recent study determined the content of different bioactive compounds in different parts of field-cultivated Pegagan harvested at different times.

## MATERIALS AND METHODS

**Study location:** The study was carried out for 9 months from January-September 2014. The experimental field was located at the University of Sisingamangaraja XII, Medan, north Sumatra situated at an altitude of 50 m above sea level.

**Seed production and field preparation:** This study used Gotu Kola seeds from the accession of Deli Serdang, north Sumatra, Indonesia due to its higher content of centellosides in a previous study by Vinolina *et al.*[17]. The mother plants were grown for two and a half months and used as the seeds. The seeds were planted in previously prepared 30 units of 30 cm raised-soil beds or plots sized 1×1 m. Each treatment (harvest time) used 10 plots where four seeds of Pegagan was planted in each plot at 40 cm distance. Randomized Block Design was used to arrange the treatments consisted of different harvest time of 56, 70 and 84 days after planting denoted as $U_1$, $U_2$ and $U_3$, respectively. Each treatment had 10 replicates to determine the effect of harvest time on biomass production of and bioactive compound in Pegagan. Fertilization was conducted at 0 days after planting (DAP) and repeated at 20 and 40 DAP using superphosphate 36 (SP36), Urea and KCL of 20, 30, and 22 g, respectively, for each plot. Field maintenance was regularly conducted to maintain the optimum growth of cultivated Pegagan.

**Harvest procedures:** The harvest times of cultivated Pegagan as the treatment variable in this current study were set at day 56, 70 and 84 after planting which were denoted as $U_1$, $U_2$ and $U_3$, respectively. Before harvest, the plots were wetted with water to facilitate easy removal of the plants. Each whole plant was taken out from the soil plots by hand. Cleaned plants were grouped according to the harvest times and part categories (leaves and roots) then weighed to determine their wet weights. Afterward, all samples were oven-dried for 3 days at 50°C and ground to powder for the subsequent bioactive compound analysis.

**Bioactive compounds in pegagan:** The powder sample harvested Pegagan (0.2 g for each sample) was prepared by pouring into a test tube, mixed with 4 mL of 90% methanol solution, shaken for 5 h. The supernatant was collected and then evaporated using a water bath set at 50°C. The dried supernatant was then mixed with 1 mL of 90% methanol solution (gradient grade for liquid chromatography), filtered using a 0.22 μM PTFE filter (Whatman membrane filter P/N E252 Buckinghamshire, England) and ready for the subsequent ultra fast liquid chromatography (UFLC) process. The analysis of bioactive compounds was performed at the Pharmacy Laboratory of the University of north Sumatera. Standard commercial asiaticoside, madecassoside and asiatic acid were obtained from Sigma Aldrich (St. Louis, MO., USA) and used to determine the retention time of each compound following the modified procedure of Jain and Agrawal[18]. The calibration of the UFLC Shimadzu DGU-20A3 followed the procedures described by Bonfill *et al.*[13] and Mangas *et al.*[19]. The retention time of asiaticoside, madecassoside and asiatic acid were determined as 18.603, 13.196, and 21.054 min, respectively. The contents of asiaticoside, madecassoside

an0d asiatic acid were determined by injecting 20 µL of the filtrates of each sample in the UFLC system with the retention times previously described. The measurement of each centelloside compound was repeated three times and the values were averaged.

**Statistical analysis:** Experimental data were analyzed using F-test in the analysis of variance (ANOVA) and significant differences among the treatments ($p<0.05$) were determined by Duncan's multiple range test (DMRT) using the SPSS 17.0 software.

## RESULTS

**Asiaticoside concentration at different harvest times:** The concentrations of asiaticoside in leaves (KAA, µg $mL^{-1}$) and roots (KAD, µg $mL^{-1}$) of Pegagan harvested at a different time ($U_1$, $U_2$, and $U_3$) are presented in Fig. 1. The figure clearly shows that both Pegagan parts contained significantly different asiaticoside amounts according to the harvest times. The highest concentration of asiaticoside was found in the leaves of Pegagan (53.55 µg $mL^{-1}$) harvested at 70 DAP which was more than four times compared to that of the leaves harvested at 56 DAP (12.52 µg $mL^{-1}$). The ratio of asiaticoside content in the leaves and roots of $U_2$ differed greatly compared to that of $U_1$ and $U_3$ ($p<0.05$).

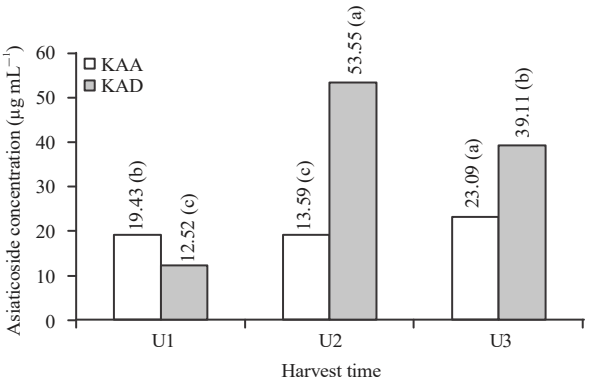


Fig. 1: Asiaticoside concentration (µg $mL^{-1}$) in leaves (KAD) and roots (KAA) of Pegagan harvested at 56 ($U_1$), 70 ($U_2$) and 84 ($U_3$) DAP. Significant differences are indicated by different letters above the bars (F-tests, $p<0.05$)

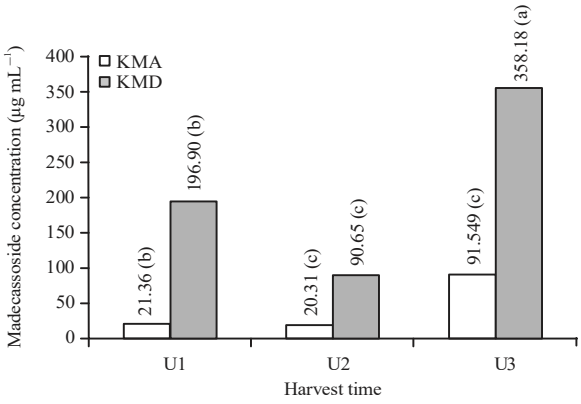


Fig. 2: Madecassoside concentration (µg $mL^{-1}$) in leaves (KMD) and roots (KMA) of Pegagan harvested at 56 ($U_1$), 70 ($U_2$) and 84 ($U_3$) DAP. Significant differences are indicated by different letters above the bars (F-tests, $p<0.05$).

**Madecassoside concentration at different harvest times:** The concentrations of madecassoside showed significant differences in different parts (leaves and roots) and harvest times (Fig. 2) ($p<0.05$). The leaves consistently had the highest concentration of madecassoside compared with the roots across different harvest times. The highest concentration of madecassoside was found in the leaves of Pegagan harvested at $U_3$ (358.18 µg $mL^{-1}$) of almost four times of the lowest concentration in the leaves harvested at $U_2$ and more than 17 times of the lowest concentration in the roots harvested at $U_2$.

**Asiatic acid concentration at different harvest times:** Results in Fig. 3 shows that the contents of asiatica acid in the leaves of Pegagan were consistently high compared that of the roots ($p<0.05$). However, there was a clear decreasing pattern of asiatic acid content in the leaves (KAAD) of Pegagan from the shortest to the longest harvest times. The content of asiatic acid in Pegagan's leaves harvested had decreased from the shortest harvest time ($U_1$ : 611.6 µ $mL^{-1}$) to less than half in Pegagan's leaves harvested at the longest time (U3 : 294.56 µ $mL^{-1}$). Despite the contents of asiatic acid in the roots (KAAA) were relatively stable at different harvest times, the F-test showed significant differences among the treatments with $U_3$ had the highest asiatic acid followed by $U_1$ and the lowest was $U_2$ ($p<0.05$).

**Dry and wet weight of harvested Pegagan at different times:** The wet weight (WW) and dry weight (DW) of Pegagan harvested at $U_1$ $U_2$, and $U_3$ and dried in an oven at 50°C for three days is presented in Table 1. The WWs and DWs of leaves and roots of harvested Pegagan at $U_3$ were the highest and $U_1$ were the lowest ($p<0.05$).

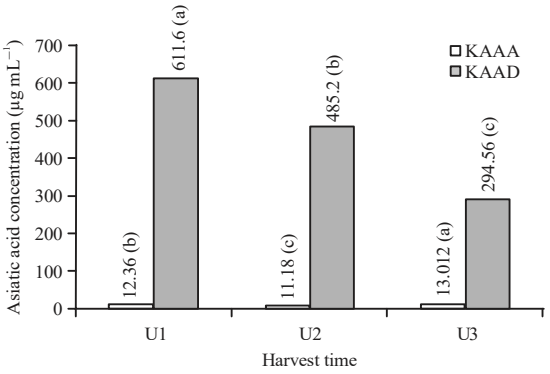


Fig. 3: Asiatic acid concentration (µg mL$^{-1}$) in leaves (KAAD) and roots (KAAA) of Pegagan harvested at 56 ($U_1$), 70 ($U_2$) dan 84 ($U_3$) DAP. Significant differences are indicated by different letters above the bars (F-tests, $p<0.05$)

Table 1: Wet weight to dry weight of leaves and roots of Pegagan harvested at 56 ($U_1$), 70 ($U_2$) and 84 ($U_3$) DAP

| Treatments | WWL (g) | DWL (g) | WWR (g) | DWR (g) |
|---|---|---|---|---|
| U1 | 77.623$^c$ | 13.665$^c$ | 66.158$^c$ | 12.291$^c$ |
| U2 | 191.206$^b$ | 17.149$^b$ | 188.894$^b$ | 22.642$^b$ |
| U3 | 201.211$^a$ | 26.206$^a$ | 200.733$^a$ | 31.553$^a$ |

Note: Different values in the same column indicated significant difference at $p<0.05$. WW(L/R): Wet Weight of Leaves/roots, DW(L/R): Dry Weight of Leaves/roots

## DISCUSSION

This current study found that there is an opposite relationship between the contents of asiaticoside-madecassoside and asiatic acid in both the leaves and roots of Pegagan cultivated at field condition. Asiaticoside-madecassoside content in whole plant of Pegagan increased over time whilst at the same time, the content of asiatic acid decreased. This finding reconfirms the findings of Kim *et al.*[11] and Bonfill *et al.*[13] who had found similar results with Pegagan cultivated under laboratory condition and treated with elicitors to increase the contents of centellosides. However, the level of centellosides in the whole plant of Pegagan found in this study was significantly higher particularly for asiaticoside which was up to five-fold higher compared to that of Bonfill *et al.*[13] but relatively similar to that of Kim *et al.*[11]. The relatively higher content of centelloside found in this study was due to the twice longer cultivation period. This longer cultivation period had allowed the build-up of the secondary metabolites in the leaves and roots of Pegagan despite no elicitors used. Similar result was also reported by Rafi *et al.*[1] that harvest time determines the concentration of centelloside which is going to be accumulated in whole plant Pegagan. However, their study did not distinguish the concentrations of different centellosides in leaves and roots of Pegagan. The field condition in which the Pegagan were cultivated is also argued to support all nutrients required by the plant compared to the more sterile and controlled laboratory condition. This was described by Rafi *et al.*[1] and Jamil *et al.*[20] as the effect of geographic location and environmental condition which also have the role the production of centellosides in Pegagan.

Furthermore, the patterns of centellosides showed a consistent trend that the leaves of Pegagan had a higher content of centellosides compared to the roots across different harvest times at field condition. A laboratory study by Kim *et al.*[11] and Mangas *et al.*[19] also found that Pegagan mainly synthesizes the secondary metabolites in leaves and an only limited amount in the roots. Similarly, Zainol *et al.*[21] had found that leaves of Pegagan has the highest concentration of centellosides compared to the root. A detailed study by Gupta *et al.*[16] also reported a high concentration of different centellosides in a similar order as found in this study. However, they studied the morphometric variation in relation to bioactive compounds of Pegagan leaves sourced from the wild population which is not comparable to this study. It can be said that accumulation of the secondary metabolites in the leaves of Pegagan is mainly for defense mechanism to predation and diseases due to its perennial nature. Rosenthal and Janzen[22] argued that secondary metabolites occur in a large amount in a particular plant part as a defense mechanism to predation and diseases. Such a defense mechanism might explain some woody plants produce a higher amount of secondary metabolites in roots compared in the leaves. For example, cassava produces cyanogenic glucosides in its cassava tubers to deter root borers[23]. This might be the case for Pegagan where the higher amount of centellosides in leaves serves as a deterrent for disease, pest and predators although another study is required to confirm this. In addition, the roots dry weight of Pegagan was relatively heavier compared to that of the leaves across different harvest times. This implied that it is more economical to harvest the leaves than the roots because leaves are easier to harvest and have less weight compared to the root. It also corresponds to the higher amount of asiaticoside and madecassoside in the leaves that can be synthesized compared to that of the roots[4,5].

Future research direction regarding the effect of harvest time on field-cultivated Pegagan can be focused on the addition of elicitors such as Methyl Jasmonate to stimulate the production of centellosides in Pegagan. The research on Methyl Jasmonate to increase centellosides in Pegagan cultivated under laboratory or controlled condition are huge

in number which show a promising result. However, its application in field-cultivated Pegagan has yet to be done.

## CONCLUSION

The study revealed that the Pegagan accession of Deli Serdang, Indonesia has asiaticoside and madecassoside in the leaves and roots which increases and asiatic acid which decreases with the passage of time. All the three centellosides are mainly found the leaves and very little in the roots across different harvest times suggesting that these secondary metabolites are for defense mechanism from pest, predation, and diseases due to the closeness of aerial parts of Pegagan to the ground.

## SIGNIFICANCE STATEMENT

This study discovered different harvest time on Pegagan can be beneficial for maximizing the level of bioactive compounds in commercial field cultivation of this plant. This study presented new findings regarding bioactive compounds level of Pegagan cultivated in field-condition and harvested at different times, which are currently lacking in the literature. This study will help researchers, farmers and biopharmaceutical industries to uncover the critical areas of harvest time settings of field-cultivated Pegagan in order to maximize bioactive compounds in the plant that many researchers and practitioners were not able to explore. Thus, a new theory on harvest time and environmental factors which affect the plant production of bioactive compounds may be arrived at.

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