Phytochemical screening and biomass production of *Centella asiatica* (L.) Urb of Samosir – Indonesia accession cultivated on acid soil with different phosphorus treatments

N S Vinolina* and R Sigalingging

1Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.
2Department of Agricultural Engineering, Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *noverita@usu.ac.id

**Abstract.** Various studies concluded that *Centella asiatica* (L.) Urb, called pegagan in Indonesia, contains bioactive compounds known to have health benefits and used to treat diseases. However, cultivation of the plant has yet to produce optimal yield. This research is a part of the effort to develop an optimal large-scale *Centella asiatica* (L.) Urb, cultivation in Indonesia. This research aimed to determine the effects of phosphorus application on the phytochemical contents and biomass production of pegagan. The experiment used a single factor with six dosage levels of P$_2$O$_5$ fertilizer arranged in a non-factorial randomized block design with three replicates. The dosages were 0 (control), 20, 40, 60, 80, 100 kg P$_2$O$_5$/ha. The application of phosphorus fertilizer affected the length growth of the primary tendril despite being cultivated in relatively acid soil condition. The phytochemical screening showed positive tests for saponin, tannin, phenolic, flavonoid, triterpenoid, steroid and glycoside in pegagan treated with and without phosphorus fertilizer. The phytochemicals were present in both leaves and roots with an exception for alkaloid. This research concludes that phosphorus treatment did not significantly affect the number of leaves, wet weight per plot, dry weight of leaves and petioles, dry weight of roots and tendrils (biomass).

1. **Introduction**

*Centella asiatica* or pegagan has been traditionally used as a medicinal plant in various cultures, particularly in Asian countries. The health benefits of pegagan are passed from generation to generation, citing that its properties could directly improve the health of people who consume it. Such a comprehensive applications, as well as health and economic benefits, create increased demand for pegagan, which is primarily sourced from China and India. Currently, China is the largest exporter of medicinal and aromatic plants, including pegagan[1]. Indonesia has the potential to cultivate and market pegagan in large scale since the plant thrives in most parts of Indonesia, including Samosir, North Sumatra, which is the site of this research. Although wild pegagan can be abundantly found in Samosir, no meaningful studies have been carried out to develop large scale farming of the plant in Samosir and North Sumatra in general.

Empirically, pegagan has been proven to accelerate wound healing, reduce inflammation, and even treat rheumatism, asthma, haemorrhoids, tuberculosis, leprosy, dysentery, fever, as well as serve as an
appetite enhancer. Pegagan contains several bio compounds such as saponin and asiaticosides [2,3]. Studies have suggested that the bioactive compounds of asiaticosides are effective to improve wound healing and are beneficial to cure leprosy and tuberculosis [4,5]. To acquire enough quantity and high quality of these compounds, improved cultivation of pegagan on a large scale needs to be developed and supported [6].

The current knowledge regarding the benefits of organic compounds revolves around specific compounds produced naturally by plants known as secondary metabolites. Certain plants used as medicines contains specific chemical compounds known as bioactive substances. Most of the bioactive compounds found in plants are usually secondary metabolic compounds such as alkaloids, flavonoids, steroids, terpenoids, saponins, and others.

Pegagan have been used not only in traditional treatments but also in modern medical applications to treat various diseases [5,7,9] leading to commercial cultivation in several countries such as China and India [10,11]. The demand for pegagan has increased significantly, which currently is supplied from the wild population and could lead to the overexploitation of this plant worldwide [12].

Past researches on the content of centelloside in pegagan have focused on the complexities and benefits of the compounds [13], their concentration in various parts of the plant [14-16] and general reviews of their growth under controlled conditions [4]. Limited literature or studies, especially in the Indonesian region, also have provided information on the effect of harvest time on the content of bioactive compounds from commercially cultivated pegagan. For example, notable differences in the quality and quantity throughout the year were observed in the production of pegagan cultivated field condition [2,3,6,17,18]. Another example is a recent finding from the study of [17] conducted in Samosir, North Sumatra, Indonesia which shows that pegagan can grow well on acid soils and still produce/contain centelloside compounds. This new finding somewhat contradicts with the contemporary knowledge that the formation of secondary metabolites is influenced by many factors, including temperature, pH, water activity, and light intensity. The thermal (non-photochemical) reaction rate is sensitive to temperature, and some reaction rates will increase with an increase in temperature of 100C. Relatively dry land, pH, and soil moisture are essential parameters for the formation of secondary metabolites in plants, including pegagan. Hence the farming location, weather pattern, and definitely plant accession play substantial roles into the inconsistency of pegagan production and deserves further study [13,17].

A previous study by Vinolina [18] argued that phosphorus application without applying methyl jasmonate produced higher biomass and increased asiaticoside content in pegagan's leaves. In accordance, the roots were also found to have a higher quantity of asiaticoside per g of dry matter [18]. Other findings of studies have also discussed the potential benefits of pegagan in fish farming as feed to increase the immune system mechanism of Nile tilapia [19], its efficacy in preventing age-related retinal deterioration and its usefulness in developing drugs or functional foods [20], protecting neuron cells [21,22]. Such importance and health benefit of pegagan have to be supported by the improvement of pegagan cultivation in commercial scale. Therefore, the objective of this research was aimed at determining the effects of phosphorus on phytochemical contents and biomass of pegagan cultivated on acid soil.

2. Materials and methods
This study used pegagan of Samosir accession as the treatment plant due to its ability to grow on relatively acid soil and showed relatively good growth. The experiment used a single factor with six dosage levels of P2O5 fertilizer arranged in a non-factorial randomized block design with three replicates. The six levels of P2O5 fertilizer doses were as follows: 0 (control), 20, 40, 60, 80, 100 kg P2O5 / ha.

2.1. Cultivation field and research preparation
The cultivation field was located in Salaon Toba Village, Ronggur Nihuta, Samosir, North Sumatra. The area was situated above 1.000 meters from the mean sea level (MSL). This study was conducted from March to Agustus 2020. Soil samples at the site were collected and tested for pH, total organic carbon
(C), nitrogen, available phosphor, and total K\textsubscript{2}O using potentiometric, Walkley-Black volumetric, Kjedhal volumetric, spectro-volumetric, and atomic absorption spectroscopic (AAS, HCl 25%) methods, respectively. All soil samples were analyzed at the Sucofindo Laboratory, North Sumatra, Indonesia. Afterwards, the field was cleared from weeds and ploughed to create 18 plots of 30 cm raised-soil bed with 1 m x 1 m in size. The distance between the plots was 0.5 meter to allow better watering and field maintenance.

2.2. Seed planting and maintenance
The stolons of pegagan of Samosir accession, North Sumatra, Indonesia were directly planted after separated from the mother plant. Each plot was planted with four stolons at 40 cm distance to each other. Urea and KCL were applied as fertilizer three times at 0, 20, and 40 days after planting (DAP). At each application of fertilizer, Urea and KCL were given 30 g/plot and 22 g/plot, respectively around the planted seeds.

Regular watering, weeding, and replanting crops were done regularly. Watering was carried out one daily in the afternoon, depending on the weather conditions. New stolons were planted to replace dead plants two weeks after the initial planting. Regular maintenance included daily manual removal of weeds. Pest and disease control were regularly conducted each week. Final harvest was carried out by taking out the whole part of the plant on the 12 weeks after planting (DAP).

2.3. Growth characteristics' measurement
The leaves were counted once per week during the 12 cultivation period. Only the fully formed and opened leaves were counted whilst dry yellowish leaves were left out.

2.4. Post-harvest handling
Harvested pegagan plants were separated into two categories. The first category consisted of leaves and petioles. The second consisted of roots and tendrils. The collected leaves were as the sample for phytochemical screening.

3. Results and discussion

3.1. Leaves (strands) growth

![Figure 1](image_url)

Figure 1. Graph of leaf number development with phosphorus treatment (F\textsubscript{0} = 0 P\textsubscript{2}O\textsubscript{5} kg / ha), (F\textsubscript{1} = 20 P\textsubscript{2}O\textsubscript{5} kg / ha), (F\textsubscript{2} = 40 P\textsubscript{2}O\textsubscript{5} kg / ha), (F\textsubscript{3} = 60 P\textsubscript{2}O\textsubscript{5} kg / ha), (F\textsubscript{4} = 80 P\textsubscript{2}O\textsubscript{5} kg / ha), (F\textsubscript{5} = 100 P\textsubscript{2}O\textsubscript{5} kg / ha).
The average number of leaves (strands) of the parent plant during the cultivation period from 1 to 12 WAP is provided in Figure 1. Based on the statistical analysis of variance, the total number of leaves from each plant during the cultivation period was not significantly affected by the phosphorus treatments. However, the collected data showed that treatment F1 and F2 had a relatively higher number of leaves compared to treatment F0.

3.2. Pegagan biomass production
The average data of pegagan plant production are listed in Table 1. Similar to previous observation on leaves, the measured WWP, DWLP, WWLP, DWLP, WWRP and DWRP values were not significantly affected by the phosphorus. However, treatment F2 (40 kg P2O5 / ha) was found to have the highest production compared to the other treatments.

**Table 1.** Average values of WWP, DWLP, DWRP of pegagan treated with phosphorus fertilizer.

| Treatment         | WWP (g)  | DWLP (g) | DWRP (g) |
|-------------------|----------|----------|----------|
| F0 = 0 kg P2O5/ha | 723.33   | 61.30    | 64.93    |
| F1 = 20 kg P2O5/ha| 1,250.00 | 78.47    | 83.03    |
| F2 = 40 kg P2O5/ha| 1,320.00 | 104.70   | 119.20   |
| F3 = 60 kg P2O5/ha| 753.33   | 63.27    | 62.57    |
| F4 = 80 kg P2O5/ha| 1,060.00 | 82.70    | 94.13    |
| F5 = 100 kg P2O5/ha| 1,083.33 | 78.33    | 92.17    |

Note: Wet Weight Per Plot (WWP), Dry Weight of Leaves and Petioles (DWLP), Dry Weight of Roots Tendrils (DWRP)

3.2.1. Wet weight per plot (g). Data on the average wet weights per plot of pegagan treated with phosphorus fertilization is provided in Table 1. The statistical analysis shows that Phosphorus fertilizer treatment did not significantly affect the wet weight values of pegagan between plots. The lowest wet weight per plot was achieved by pegagan treated with 0 kg P2O5/ha (without phosphorus fertilization) (± 723.33 g), and the highest was obtained by pegagan treated with 40 kg P2O5/ha (± 1320.00 g).

3.2.2. Leaves and petioles dry weight (g). The average dry weight of leaves and petioles is listed in Table 1. The lowest dry weight of leaves and petioles per plot was obtained by pegagan treated without phosphorus fertilizer or 0 kg P2O5/ha (± 61.30 g), and the highest was obtained by pegagan treated with 40 kg P2O5/ha (± 104.70 g).

3.2.3. Roots and tendrils (g) dry weight. The average dry weight values of roots and tendrils are provided in Table 1. The highest dry weight values of roots and tendrils of pegagan per plot were obtained in the phosphorus fertilizer treatment of 40 kg P2O5/ha (± 104.70 g).

3.3. Screening of phytochemical contents
The screening of phytochemical contents was performed only for the samples of leaves which is provided in Table 2.

The highest measured growth parameters of pegagan (number of leaves, wet weight per plot, leaf dry weight, and root dry weight, the highest) were found in the treatment of phosphorus at the level of 40 kg P2O5 / ha. These results suggest that phosphorus application and the availability of nutrients in the soil are sufficient to support the growth of pegagan. Phosphorus is one of macronutrients needed by plants. It plays an essential role in various life processes, such as photosynthesis, carbohydrate metabolism, and energy flow processes in plants.

A previous study shows that phosphorus content of soil influenced the centelloside content of pegagan [17]. The role of phosphorus in plants is to stimulate better root formation [23], which is responsible for increasing water and nutrients absorption. In addition, chlorophyll concentration in leaves also increases with the increase of phosphorus application which in turn could boost
photosynthesis process to produce photosynthate leading to the increase of asiaticoside content production [24,25]. Phosphorus remains in plant's tissue in the forms of free phosphate or organically-bonded esters. The plant forms phosphate esters by metabolizing alcohols, sugars, acids and polyphosphates. This energy-rich compound is argued to be an intermediate compound for the pentose phosphate pathway of primary metabolites and derived from precursors to secondary metabolites [24].

**Table 2.** Phytochemical screening in leaves pegagan of Samosir accession.

| Phytochemical | Phytochemical Content |
|---------------|-----------------------|
|               | F₀ | F₁ | F₂ | F₃ | F₄ | F₅ |
| Alkaloid      | -  | -  | -  | -  | -  | -  |
| Saponin       | +  | +  | +  | +  | +  | +  |
| Tannin        | +  | +  | +  | +  | +  | +  |
| Phenolic      | +  | +  | +  | +  | +  | +  |
| Flavonoid     | +  | +  | +  | +  | +  | +  |
| Triterpenoid  | +  | +  | +  | +  | +  | +  |
| Steroid       | +  | +  | +  | +  | +  | +  |
| Glycoside     | +  | +  | +  | +  | +  | +  |

The screening analysis for phytochemical contents of *Centella asiatica* had found saponin, triterpenoid, tannin, flavonoid, phenolic, steroid, and glycoside in the leaves of pegagan. However, alkaloid compounds were not found in the screening analysis. The content of saponins in pegagan is similar to that of saponin in other plants and commonly used as the primary compound in the production of pesticides, detergents and organic molluscsicides products as well as medical products for human and animal. Furthermore, the existence of terpenoids and tannins in pegagan is promising because they can be used for anti-inflammatory and analgesic compounds. In many plant species, tannins serve as antimicrobial compounds. For human health, tannins application could accelerate wound healing process and reduce mucous membrane inflammation. Tannins have the potential application in cancer treatments (anticarcinogen), heart diseases protections (cardiovascular), and general health improvements such as antiapoptosis and antiaging.

Pegagan also shows to have flavonoids content. Plants are known to produce flavonoids in response to microbial infection. Flavonoids have biological properties similar to that of antimicrobial substances which could deter a wide array of pathogenic microorganism [26]. In addition, glycosides present in pegagan is also effective to treat high blood pressure in hypertension patients [26]. This current research also found that pegagan has triterpenoid compounds. Triterpenoids are classified as the derivative compounds of primary metabolite precursors bio-synthesized through the mevalonate pathway. The process will then produce geranyl pyrophosphates which are the primary metabolites responsible in the formation of monoterpenoids and their derivatives.

On the other hand, farnesyl pyrophosphate is responsible for increasing sesquiterpenoids and converting squalene to triterpenoids. Furthermore, farnesyl diphosphate synthase plays an essential regulatory role in cell and tissues development of plants. This compound is argued to serve as one of the main regulatory enzymes in triterpene bio-synthesis [27]. This bio-synthesis occurs the leaves of pegagan in which the asiaticoside is produced and content increases over time [5].

In this research and others, the application of phosphorus had affected the levels of asiaticoside, madecasoside and asiatic acid in different pegagan accessions. Bio-synthesis of centellosides through the mevalonic pathway in the cytoplasm produces mevalonate hydroxymethyl glutaryl-CoA which in turn leads to the formation of the common precursor of all terpenoids, namely 5-carbon isopentenyl diphosphate. Centellosides in pegagan consist of asiaticosides, madecasosides, asiatic acids and madecacic acids which are categorized as saponin ursane [2,3,17]. The production of these compound
could be accelerated by phosphorus availability. Phosphorus has been termed as an essential element of various phosphate sugars. Phosphorus has multifunction in plant's respiration, photosynthesis, and other metabolic processes. Phosphorus also significantly affect the function of energy storage and transfer, increase the production of pegagan biomass as well as the harvested dry mass and quality of pegagan [23]. Considering the importance of pegagan as medicinal herbs and the limited availability of large scale pegagan cultivation, it is vital to carry out further studies to determine the response of pegagan and the production of centellosides to various external factors such as other types of fertilizers or other improved farming practices.

4. Conclusions
This recent research confirms that the cultivated pegagan (Centella asiatica) of Samosir accession treated with and without phosphorus applications showed positive phytochemical screening tests for saponin, triterpenoid, tannin, phenolic, steroid, flavonoid, and glycoside. The phytochemical compounds were found in the leaves despite negative for alkaloid compound. Phosphorus applications did not significantly affect the number of leaves, total wet weight, leaves and petiole dry weight, roots and tendrils dry weight (biomass) of pegagan cultivated in acid soil conditions. However, the increased tendency of those growth parameters was observed on phosphorus treatment (F2: 40 kg P2O5/ha) showing the highest values compared to the other treatments.

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