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Research Article

Genetic Diversity and Phytochemical Analysis of *Centella asiatica* in Central Java: A Study Based on Electrophoretic Profiling and Environmental Factors

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Abstract

This study presents a comprehensive exploration into the genetic diversity and chemical composition of *Centella asiatica*, a medicinal plant of significant pharmacological interest, harvested from three distinct locales within the rich biodiversity hotspot of Central Java, Indonesia. Through meticulous analysis, we scrutinized a total of sixteen samples, employing advanced techniques such as genomic DNA extraction and electrophoretic profiling. Our findings unveil a diverse array of banding patterns across the sampled populations, indicative of substantial genetic variations within *Centella asiatica*. Concurrently, quantitative assessments of saponin and terpenoid content provide invaluable insights into the biochemical richness of these plant specimens. Moreover, environmental parameters including temperature, soil pH, humidity, and lux meter readings were meticulously recorded, facilitating a comprehensive understanding of their influence on the plant's phytochemistry. This interdisciplinary approach allows for a nuanced exploration of Centella asiatica genetic makeup, chemical constituents, and environmental interactions, providing a solid foundation for further research into its therapeutic potential and ecological significance.

Keywords: *Centella asiatica*; Genetic Diversity; Electrophoresis; Saponins; Terpenoids; Environmental Factors

Introduction

Background

Genetic diversity and chemical composition analysis

This study investigated the genetic diversity and chemical composition of **Centella asiatica** harvested from three distinct regions in Central Java, Indonesia. Sixteen samples were analyzed using genomic DNA extraction and electrophoretic profiling, revealing significant disparities in banding patterns [1], suggesting variations in the genetic makeup of **Centella asiatica** across the study regions. The study also quantified the levels of saponin and terpenoid content, key bioactive compounds known for their therapeutic properties [2], while considering environmental factors potentially influencing the plant's phytochemistry. This research aims to uncover the intricate relationship between **Centella asiatica**'s genetic makeup, chemical constituents, and environmental influences [3], which is crucial for comprehending the plant's therapeutic potential in diverse geographical contexts.

Traditional medicinal uses of *Centella asiatica*

The backdrop of this study lies in the rich history of *Centella asiatica* medicinal applications, deeply ingrained in traditional medicine for its myriad health benefits [4]. Recognized for its anti-inflammatory properties, wound healing efficacy, and cognitive enhancement attributes, *Centella asiatica* serves as a focal point for pharmacological exploration [7]. Leveraging insights from recent studies, particularly regarding the influence of environmental factors on phytochemical composition, this investigation aims to contribute to the ongoing discourse on harnessing the plant's thera-

peutic potential [4]. By examining genetic variability and chemical makeup across different regions, the study seeks to provide a nuanced understanding of *Centella asiatica*'s pharmacological profile and its implications for pharmaceutical and cosmetic industries [1].

Through a meticulous analysis of *Centella asiatica* samples from three distinct locales in Central Java, Indonesia. This investigation seeks to unravel the intricate relationship between genetic diversity, chemical composition, and environmental influences [2]. Rooted in the plant's storied history of medicinal use, particularly in traditional medicine for its anti-inflammatory, wound healing, and cognitive enhancement properties, this study aims to contribute to the ongoing dialogue surrounding its therapeutic potential [5]. Drawing from recent research highlighting the impact of environmental factors on phytochemical composition. The study endeavors to offer insights into *Centella asiatica* adaptability and resilience across different geographical contexts [7]. By examining genetic variability and chemical makeup, the research seeks to provide a comprehensive understanding of *Centella asiatica*'s therapeutic efficacy and its implications for pharmaceutical and cosmetic applications [2].

Interdisciplinary exploration: Genetics, chemistry, and environment

This investigation into the genetic and chemical makeup of *Centella asiatica* sourced from Central Java, Indonesia. Is deeply rooted in the plant's historical significance in traditional medicine [5]. Recognized for its diverse medicinal properties, including anti-inflammatory, wound healing, and cognitive enhancement attributes, *Centella asiatica* serves as a focal point for pharmacological exploration [6]. By leveraging insights from recent scholarly works, particularly focusing on environmental influences on phytochemical composition, this study aims to offer a nuanced understanding of *Centella asiatica*'s therapeutic potential [3]. Through the development of innovative oral drug delivery systems, such as chitosan-embedded liposomes incorporating *Centella asiatica* extract, the research seeks to advance therapeutic interventions, particularly in addressing conditions like Alzheimer's disease [7].

The investigation into *Centella asiatica* genetic diversity and chemical composition is significant within the context of its traditional medicinal use and the challenges posed by environmental factors [8]. With a focus on understanding the intricate relationship between genetic variability, chemical constituents, and environmental influences, this research contributes to the broader discourse on herbal medicine [9]. By quantifying saponin and terpenoid levels and analyzing environmental parameters, the study aims to elucidate the factors governing *Centella asiatica* therapeutic potential [5]. Through this exploration, the research endeavors to provide valuable insights into optimizing the plant's medicinal applications in both traditional and modern contexts [2].

Implications for therapeutic development and industrial applications

Centella asiatica, revered for its medicinal properties, serves as a focal point for this investigation into genetic diversity and chemical composition [4]. With its traditional applications in addressing various health concerns, including inflammation, wound healing, and cognitive enhancement, *Centella asiatica* holds promise for pharmacological exploration [10]. By delving into the genetic variability and chemical makeup of *Centella asiatica* sourced from Central Java, Indonesia, this study aims to deepen our understanding of its therapeutic potential [3]. Drawing on recent research on environmental influences, particularly regarding phytochemical composition, the investigation seeks to provide insights into optimizing the plant's medicinal applications [11]. Through this interdisciplinary approach, the research aims to contribute to the advancement of herbal medicine and pharmaceutical development [4].

Objectives

- To analyze the genetic diversity of *Centella asiatica* from different locations in Central Java using electrophoretic profiling.
- To quantify the saponin and terpenoid content in the plant's extracts.
- To examine the relationship between environmental factors (temperature, soil pH, humidity) and the phytochemical composition of *Centella asiatica*.

Methods

Selection of collection sites

The study focused on the collection of *Centella asiatica* from three distinct locations within the Tawangmangu region of Central Java, Indonesia, to capture the ecological diversity of the species. The first site, Bukit Sekipan, is situated in the highlands and characterized by a unique soil composition of clayey loam with high or-

Figure 1: Taman Hutan Rakyat near Surakarta.

ganic matter. The climate in this area is cool and humid, providing optimal conditions for the growth of *Centella asiatica*. The second site, Kemuning, is a rural area on the outskirts of Tawangmangu. This transitional environment features diverse land uses, vegetation cover, and soil types, contributing to the ecological niches that support the diversity of *Centella asiatica* strains. The third site, Taman Hutan Rakyat (Figure 1), is a natural forest habitat located near Surakarta, approximately 25 kilometers north of the city. This site is characterized by minimal human interference, dense canopy cover, high humidity, and moderate temperatures, making it an ideal environment for *Centella asiatica* [12].

Criteria for specimen selection

To ensure a comprehensive genetic representation of *Centella asiatica* populations, samples were selected based on specific criteria. Only flowering and fruiting plants were chosen to capture the genetic diversity associated with reproductive maturity. Each plant was visually inspected to ensure it was free from disease or stress, selecting only healthy specimens. To minimize the effects of clonal propagation, samples were collected from different locations and populations within each site. This approach aimed to increase the genetic diversity of the collected samples.

Collection and preservation of plant specimens

Plant specimens of *Centella asiatica* were collected from various parts of the designated sites throughout Surakarta. The specimens were identified based on morphological traits, and several environmental parameters, including light intensity, pH, humidity, and temperature, were recorded at the time of collection. The collected plant samples were preserved at the Faculty of Mathematics and Natural Sciences, Sebelas Maret University (UNS), ensuring proper temperature conditions for future reference [13].

Isolation of genomic DNA

The aerial parts of *Centella asiatica* were ground into a fine powder using a pestle and mortar. Genomic DNA was extracted from 50 mg of the powdered leaf samples using the Genomic DNA Mini Kit (Plant) in accordance with the manufacturer's instructions, we made 16 samples for the three sites. The extracted DNA's quantity and quality were evaluated using agarose gel electrophoresis. Specifically, the DNA samples were run on a 0.8% (w/v) agarose gel in 1X TBE buffer containing 1X SERVA DNA Stain G solution (SERVA Electrophoresis GmbH, Germany). Electrophoresis was conducted for 40 minutes at 100 V, and gel documentation. The genomic DNA was then stored at -20°C for future use [14].

Preparation of ethanol extracts

To prepare ethanol extracts, the roots, stems, and leaves of *Centella asiatica* were first dried in an oven to remove moisture. The dried plant parts were then ground into a fine powder using a mechanical mill. Ten grams of each powdered plant part were soaked in 100 ml of absolute ethanol for 72 hours to facilitate the extraction of bioactive compounds. After soaking, the extracts were filtered through Whatman filter paper No. 1 to remove solid residues. The filtrates were evaporated to dryness at room temperature and stored at 5°C for further analysis [15].

Quantitative analysis of saponins

The saponin content of *Centella asiatica* extracts was determined using the method described by Obadoni and Ochuko (2001). Three samples were analyzed for each site: Bukit Sekipan (B), Kemuning (A), and Taman Hutan Rakyat (C). Four grams of dried extract were combined with 20 ml of 20% aqueous ethanol and heated in a hot water bath at 55°C for 4 hours with constant stirring to ensure optimal extraction. Following heating, the extract was filtered to separate the liquid extract from the solid residue. The residue was re-extracted with an additional 40 ml of 20% ethanol to ensure complete extraction of saponins. The combined extracts were concentrated in a water bath at 90°C to a final volume of 40 ml. The concentrated extract was then subjected to a series of purification steps, including extractions with diethyl ether and n-butanol, to isolate the saponins. The final purified saponin extract was evapo-

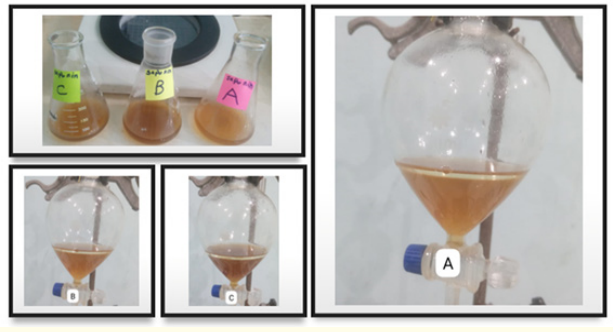

Figure 2: Methodology Overview: A systematic approach to saponin extraction from Centella asiatica using Obadoni and Ochuko (2001) method.

rated to dryness, and the dried samples were oven-dried to constant weight. The saponin content was calculated as a percentage of the initial 4 grams of dried extract used [16].

Quantitative analysis of terpenoids

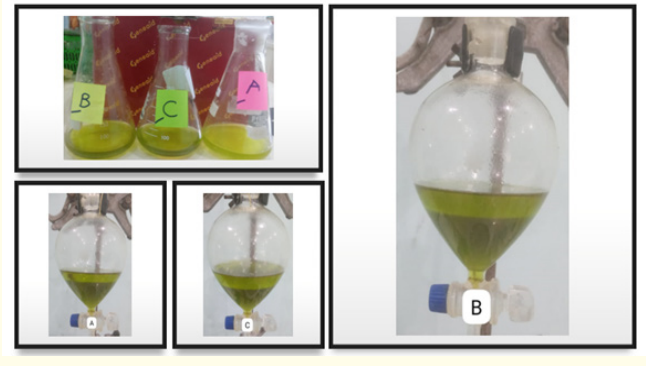

Figure 3: Determine total Terpenoids Using Ferguson (1956) Method.

The total terpenoid content of *Centella asiatica* extracts was determined using the method established by Ferguson (1956). Three samples were analyzed for each site: Bukit Sekipan (B), Kemuning (A), and Taman Hutan Rakyat (C). Four grams of dried extract were soaked in alcohol for 24 hours to facilitate the extraction of terpenoids. After the soaking period, the extract was filtered to separate the liquid filtrate from the solid residue. The filtrate was then extracted with petroleum ether to isolate the terpenoids from other components of the extract. The resulting ether extract, containing the terpenoids, was quantified as needed for further analysis [17].

Environmental factor measurements

To understand the impact of environmental factors on the chemical composition of *Centella asiatica*, parameters such as temperature, soil pH, and humidity were measured at each collection site using calibrated instruments. Measurements were recorded at standard time intervals. Statistical analyses, including correlation analysis and regression modeling, were performed to relate these environmental variables to the chemical composition of *Centella asiatica* extracts.

Results

Genetic analysis

To delve into the genetic diversity of *Centella asiatica*, DNA was meticulously extracted and analyzed from a total of sixteen samples procured from three distinct locales: Kemuning, Bukit Sekipan, and Taman Hutnan Rakayat. Each sample was meticulously labeled with unique identifiers such as KK, K2, KY, K1, K-, K., SO, S2, S1, S-, S., TO, T2, TY, T-, and T to facilitate differentiation. Through electrophoretic profiling of the extracted DNA, a fascinating array of banding patterns emerged, delineating significant genetic variations across the sampled populations.

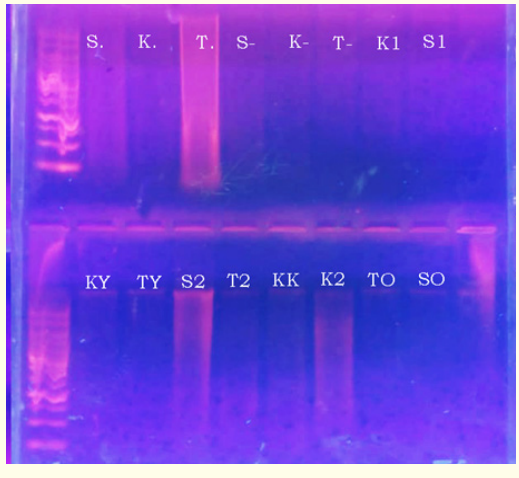

Figure 4: Exploring *Centella asiatica* Genetic Diversity Through Banding Patterns.

Of note were the samples T., S., S2, and K2, which exhibited conspicuous bands, indicative of heightened genetic diversity within these populations. In stark contrast, other samples displayed fewer or no discernible bands, signaling comparatively lower genetic heterogeneity. This striking disparity in banding patterns underscores

the nuanced genetic makeup of *Centella asiatica* populations thriving in diverse habitats. These findings not only shed light on the intricate interplay between genetic diversity and environmental factors but also underscore the plant's remarkable adaptability to varying ecological niches.

Chemical composition analysis

The investigation into the chemical composition of *Centella asiatica* extracts served as a pivotal aspect of this study, aiming to unravel the intricate nuances of its bioactive constituents. Through a rigorous quantitative analysis, the concentrations of saponins and terpenoids were meticulously determined across samples procured from the three distinct sites under scrutiny.

Site	Saponin (mg/4g)	Terpenoid (mg/4g)
Bukit Sekipan	12	22
Kemuning	27	140
Taman Hutan Rakyat	32	160

Table 1: Chemical composition variation of *Centella asiatica* across habitats: Saponin and terpenoid concentrations in milligrams per 4 grams.

Beginning with Bukit Sekipan, the measured saponin content (Table 1) stood at 12 mg/4g, while the terpenoid content was observed at 22 mg/4g. This modest concentration hints at the biochemical makeup of *Centella asiatica* specimens thriving in this locale. In contrast, the samples from Kemuning unveiled a notable uptick in saponin concentration, reaching 27 mg/4g, accompanied by a striking elevation in terpenoid levels, soaring to 140 mg/4g. Such pronounced biochemical signatures hint at the potential potency of *Centella asiatica* extracts sourced from this habitat.

However, it was the samples harvested from Taman Hutan Rakyat that truly captured attention, boasting the highest concentrations of both saponins and terpenoids among the three sites under investigation. With saponin levels measured at 32 mg/4g and terpenoids soaring to 160 mg/4g, the biochemical richness of *Centella asiatica* specimens thriving in this pristine environment is undeniable. Such significant disparities in chemical profiles across habitats underscore the dynamic interplay between environmental factors and phytochemical composition, potentially influencing the therapeutic efficacy and pharmacological properties of *Centella asiatica* extracts.

These findings not only deepen our understanding of the intricate biochemical makeup of *Centella asiatica* but also hold immense promise for unlocking its therapeutic potential in diverse medicinal applications. By elucidating the chemical intricacies of this revered botanical species, this study paves the way for future research endeavors aimed at harnessing the full spectrum of its pharmacological benefits.

Environmental parameters

Site	pH	Lux Meter	Humidity (%)	Temperature (°C)
Bukit Sekipan	7	430	86	21
Kemuning	7	6443	75	25
Taman Hutan Rakyat	7.1	750	60	26

Table 2: Variation in environmental parameters across *Centella asiatica* habitats: pH, lux, humidity, and temperature readings.

Environmental parameters, including pH, lux meter readings, humidity, and temperature, were assessed at each collection site to understand their influence on the growth and chemical composition of *Centella asiatica*. Bukit Sekipan, characterized by a pH of 7, lux meter reading of 430, humidity of 86%, and temperature of 21°C, represents a moderately humid and cool environment. In contrast, Kemuning exhibited a lux meter reading of 6443, indicative of higher light intensity, coupled with a pH of 7, humidity of 75%, and temperature of 25°C. Taman Hutan Rakyat displayed similar pH levels to the other sites but had a lux meter reading of 750, humidity of 60%, and temperature of 26°C, suggesting slightly lower humidity and higher temperature. These environmental variations may contribute to the observed differences in chemical composition among *Centella asiatica* populations, highlighting the importance of habitat characteristics in shaping plant physiology and secondary metabolite production.

Discussion

Genetic diversity analysis

The investigation into the genetic diversity of *Centella asiatica* via electrophoretic profiling of DNA extracted from samples gathered across three distinct locales within Central Java has yielded profound insights. Through meticulous analysis, significant variations in banding patterns have surfaced, shedding light on the intricate genetic makeup of *Centella asiatica* populations thriving in diverse habitats. Particularly noteworthy are the samples collected from Taman Hutan Rakyat, where conspicuous bands indicative of heightened genetic diversity were observed, starkly contrasting with samples from other locales characterized by comparatively lower genetic heterogeneity. These findings underscore the complex interplay between genetic diversity and environmental factors, emphasizing the remarkable adaptability of *Centella asiatica* to various ecological niches. This in-depth genetic analysis not only enriches our understanding of the species' genetic landscape but also reinforces the study's primary objective of elucidating the genetic diversity of *Centella asiatica* across distinct locations

in Central Java, thereby contributing significantly to the broader discourse on plant genetics and adaptation.

Phytochemical composition analysis

The quantitative analysis of saponin and terpenoid content in *Centella asiatica* extracts sourced from Bukit Sekipan, Kemuning, and Taman Hutan Rakyat unveiled noteworthy disparities in biochemical profiles. While Bukit Sekipan exhibited modest concentrations of both compounds, samples from Kemuning showcased a notable uptick in saponin concentration, coupled with a striking elevation in terpenoid levels. However, it was the samples from Taman Hutan Rakyat that stood out, boasting the highest concentrations of both saponins and terpenoids among the sites under investigation. These findings underscore the dynamic interplay between environmental factors and phytochemical composition, potentially influencing the therapeutic efficacy and pharmacological properties of *Centella asiatica* extracts. This chemical composition analysis aligns with the study's objective of quantifying the bioactive constituents in *Centella asiatica* extracts across different habitats.

Relationship between environmental factors and phytochemical composition

The assessment of environmental parameters, including pH, lux meter readings, humidity, and temperature, at each collection site provided valuable insights into their influence on the growth and chemical composition of *Centella asiatica*. Variations observed among sites likely contribute to the observed differences in saponin and terpenoid concentrations, highlighting the importance of habitat characteristics in shaping plant physiology and secondary metabolite production. These findings underscore the significance of understanding the relationship between environmental factors and phytochemical composition to optimize the medicinal applications of *Centella asiatica*. This analysis aligns with the study's objective of examining the influence of environmental factors on the phytochemical composition of *Centella asiatica*.

Conclusion

In conclusion, our comprehensive investigation delves deep into the intricate dynamics of genetic diversity, phytochemical composition, and environmental factors shaping *Centella asiatica* populations in the Central Java region. By meticulously analyzing 16 samples sourced from three distinct locales—Kemuning, Bukit Sekipan, and Taman Hutan Rakyat—we have unveiled a rich tapestry of genetic variations through electrophoretic profiling of DNA. The distinct banding patterns observed highlight the significant genetic diversity inherent within these populations. Particularly striking are the samples from Taman Hutan Rakyat, which exhibit conspicuous bands indicative of heightened genetic diversity compared to counterparts from other locales. This finding underscores the remarkable adaptability of *Centella asiatica* to thrive in diverse habitats, aligning closely with our overarching objective of unraveling genetic diversity across varied geographical settings. Through our meticulous genetic analysis, we not only shed light on the intricate genetic makeup of *Centella asiatica* but also lay a robust foundation for future investigations into its evolutionary dynamics and adaptive strategies.

Furthermore, our investigation into the phytochemical composition of *Centella asiatica* extracts uncovered notable variations in saponin and terpenoid concentrations. Specifically, samples from Kemuning and Taman Hutan Rakyat exhibited elevated levels of both compounds compared to Bukit Sekipan. For instance, the saponin content in Taman Hutan Rakyat samples measured at 32 mg/4g, while terpenoid levels soared to 160 mg/4g, underscoring the biochemical richness of specimens in this habitat. These findings highlight the potential potency of *Centella asiatica* extracts sourced from specific locales and emphasize the dynamic interplay between environmental factors and phytochemical composition. The variations observed in chemical profiles across habitats underscore the need to consider environmental influences in optimizing the medicinal applications of *Centella asiatica*, thus supporting our objective of quantifying bioactive constituents across different habitats.

Moreover, our assessment of environmental parameters provided valuable insights into their influence on *Centella asiatica* growth and chemical composition. Variations in pH, lux meter readings, humidity, and temperature among sites likely contribute to differences in phytochemical concentrations. For example, the lux meter reading in Kemuning was notably higher compared to Bukit Sekipan and Taman Hutan Rakyat, indicating higher light intensity. This environmental variation may have contributed to the observed differences in saponin and terpenoid concentrations among habitats. Our findings underscore the importance of understanding the relationship between environmental factors and phytochemical composition in shaping plant physiology and secondary metabolite production. This analysis aligns with our objective

of examining the influence of environmental factors on the phytochemical composition of *Centella asiatica* and provides valuable insights for optimizing its medicinal applications.

In essence, our study contributes significantly to the broader understanding of plant genetics, phytochemistry, and environmental influences. By unraveling the genetic diversity and chemical intricacies of *Centella asiatica*, we pave the way for future research endeavors aimed at harnessing its full pharmacological potential in diverse medicinal applications.

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