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FERN SPECIES DISTRIBUTION AND ITS PHYSIOLOGICAL PERFORMANCE AT HUTAN UiTM CAWANGAN NEGERI SEMBILAN

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Abstract

Ferns have a long geological history earliest back millions of years, and they can be found in a variety of environments around the world. This makes them one of the oldest groups of vascular plants on the planet. Ferns played a significant role in shaping the Earth's flora during this ancient period, evolving alongside other plant species and helping to form complex ecosystems. The loss of biodiversity can impair the provision of important ecological services to humans. Hutan UiTM Cawangan Negeri Sembilan has a humid climate and a high potential for pteridophyte growth. There is a lack of knowledge regarding fern diversity, and this study will provide more information about fern diversity. The purpose of this research is to study the distribution of fern species and their physiological performance at Hutan UiTM Cawangan Negeri Sembilan. The study revealed 8 families of ferns that represent 8 genera and 9 species, which include Lygodiaceae, Gleicheniaceae, Pteridaceae, Thelypteridaceae, Selaginellaceae, Osmundaceae, Lycopodiaceae and Polypodiceae. The highest species that was found is *Selaginella willdenowii* which has 51 individuals and the lowest species that were found is *Lycopodiella cernua* which has only 3 individuals. The Shannon-Wiener Index was H'=1.95 and the Pielou Evenness Index was J'=0.89. Our findings indicate that relative chlorophyll content is an important measure for characterizing plant nitrogen nutritional status. The highest average of relative chlorophyll content recorded is 52.07 ± 2.18^{a} which is *Osmunda vachellii* and the lowest is 10.63 ± 2.89^{f} which is *Dicranopteris linearis*. Overall, the findings suggest that ferns have high biological significance because they have a primitive life cycle with two discrete and more or less independent generations. This study will benefit ecosystems where ferns exist by providing shelter, shade, erosion protection, chemical absorption and microhabitats that benefit other species.

Keywords: Fern diversity, fern physiology

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Introduction

Ferns are seedless vascular plants in the plant kingdom Pteridophytes. Their body structure, like that of other vascular plants, is divided into roots, stems, fronds, and pinna. This plant group is cryptogamic because it does not produce flowers, seeds, or fruits, which distinguishes it from higher plants. They share the same mode of reproduction as bryophytes and algae but differ in that they have vascular tissue (Yusuf, 2010). The majority of ferns are found in low altitude tropical forests, but they may also colonize the understory of some temperate forests. There are approximately 4400 species of ferns recorded in Southeast Asia, with 1165 species found in Malaysia's tropical rainforests.

Fern ecology has evolved from simple observations of fern habitat characteristics to long-term studies of their complex functions in nutrient cycling and successional dynamics of natural ecosystems. As ferns become more significant in the horticulture trade and as invaders of damaged habitats, recent human activities have required a greater understanding of their ecological role (Sharpe et al., 2010). Ferns are used by humans in food, medicine, agriculture, and horticulture. For example, in the United

States, unfurled croziers (immature leaves) of *Matteuccia struthiopteris* (ostrich fern) are consumed. In medicine, the lycophyte *Huperzia serrata* (Chinese club-moss) produces an alkaloid that has proven beneficial in the management of epilepsy.

Biodiversity provides a wide range of ecological services that are critical to human well-being both today and in the future (Dai et al., 2020). Human disturbance is a serious threat to the biodiversity of vascular plants. Patterns of fern diversity versus disturbance gradients, including taxonomic, phylogenetic, and functional diversity features, have received less attention than seed plants. The loss of biodiversity can impair the provision of important ecological services to humans. As a result, fern distribution is critical for more sustainable ecosystem management in the future. The importance of studying both distribution and physiological performance develops from ferns' ability to serve as bioindicators of ecosystem health. Bioindicators are species that reflect the whole condition of an ecosystem and serve as sensitive indicators of changes in the environment. Understanding where ferns thrive and analyzing their physiological responses allows researchers to identify connections between these findings and the overall health of the ecosystem. For example, declines in fern abundance or signs of physiological stress may indicate environmental disturbances or changes that could have an impact on the forest's overall biodiversity and ecosystem balance.

Ferns have high biological significance because they have maintained a primitive life cycle with two distinct and more or less independent generations, or growth phases, with plants that are completely different in many ways (Christenhusz & Chase, 2014). Ferns are biologically significant because they maintain a primitive life cycle with two distinct generations: gametophytes and sporophytes. The gametophyte, which is often hidden and rooted in the soil, produces gametes that, when fertilized, give rise to the sporophyte, the more visible fern plant. This unique generational alternation helps to increase genetic diversity in fern populations, which is important for adaptation in changing environments. The alternation of generations in ferns has important implications for their biological functions. Ferns' ecological and evolutionary significance is highlighted by their ability to maintain a primitive life cycle with two distinct generations. Their contribution to genetic diversity, ecological interactions, and plant evolution emphasizes the importance of preserving fern populations and understanding their role in terrestrial ecosystems. Thus, this study will benefit ecosystems where ferns exist by providing shelter, shade, erosion protection, chemical absorption and functional microhabitats for other species. Aside from improving aesthetics, they also help to remove harmful air pollutants from the environment which will increase humidity and aid in restoring humidity naturally.

This study was conducted to determine the distribution of fern species found at Hutan UiTM Cawangan Negeri Sembilan, as well as to investigate their physiological performance, with direct application to soil erosion control and ecosystem health assessment. The findings can be used to develop informed conservation strategies and long-term forest management practices in Hutan UiTM Cawangan Negeri Sembilan.

Methods

Study Area

This study was conducted at Hutan UiTM Cawangan Negeri Sembilan (Figure 1) and continued in the Plant Physiology laboratory at UiTM Kuala Pilah from March 2023 until August 2023.

Figure 1. Map's location of Hutan UiTM Cawangan Negeri Sembilan

Methodology

This study was obtained to determine the distribution patterns of fern species in Hutan UiTM Cawangan Negeri Sembilan while also investigating their physiological performance. The goals were to gain a thorough understanding of the spatial distribution of fern species in the designated forest area, as well as to investigate the physiological aspects of these ferns. The study's dual-pronged approach aimed to provide insights into the ecological preferences and adaptability of ferns in this specific ecosystem, thereby contributing valuable information for biodiversity conservation and ecosystem management.

The methodology used in this study are sampling site, samples collection, data collection of species identification, measurement of relative chlorophyll content using SPAD-502 meter and statistical analysis. Figure 2 shows the flowchart of methodology used in this study.

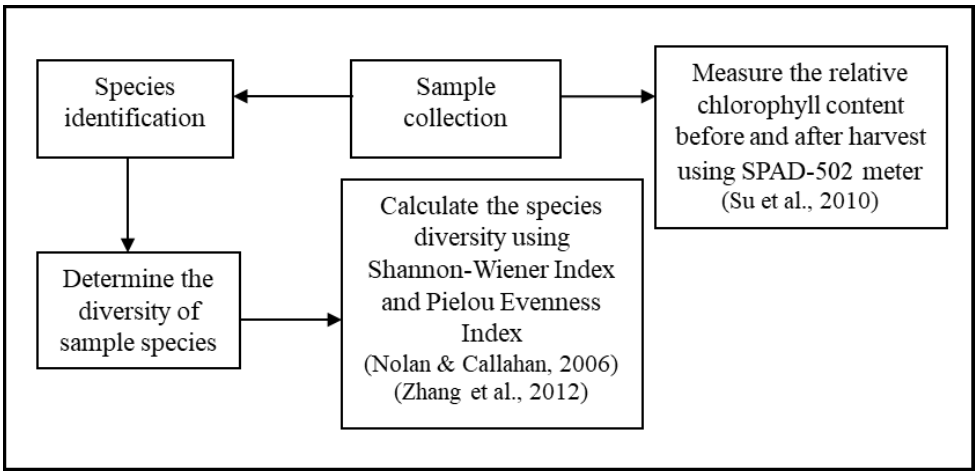


Figure 2. Methodology used in this study

Sampling site and samples collection

The collection of fern specimens was collected at Hutan UiTM Cawangan Negeri Sembilan. A non-random sampling method that has been adopted where the area is a priority to ensure that at least one individual fern is present in that area (Akinsoji et al., 2016). Only structurally complete specimens with rhizome, stalk, frond, and spores were collected. The specimens chosen were in good condition, with no insect-damaged plants. The plant specimens were collected with a shovel to the base, then placed in plastic bags and secured with plastic ropes. Following the collection process, the specimens were immediately cleaned by draining them in tap water to remove all root dirt.

Data collection of species identification

Ferns are distinguished from other plant groups by their unique reproductive system, which alternates between gametophyte and sporophyte generations. Unlike seed-bearing plants, ferns reproduce via spores, indicating a more primitive life cycle. Ferns are extremely adaptable, thriving in a wide range of ecosystems around the world, including tropical rainforests, temperate woodlands, arid deserts, and alpine regions. Ferns thrive in shaded and moist conditions, adding significantly to understory vegetation and demonstrating resilience in disturbed habitats. Their widespread distribution highlights their ecological versatility, emphasizing their critical role in maintaining biodiversity and ecological balance across a variety of terrestrial landscapes.

The root type, structure, surface condition and size of the rhizome section were all observed. Leaf morphology will include leaf shape, leaf area, pinnate or not pinnate, leaf arrangement and leaf apex. The spores from the samples were also found to play a significant role in distinguishing the fern species by observing their arrangement and shape (Nazihah et al., 2018). As a result, all of these factors have been measured in order to properly identify the species of sample and analyze it. The method that has been used to identify the type of each species of fern is to refer to the book (Xu & Deng, 2017). The fern species' character and structure must correspond to the literature and images in the reference books.

Determination of species diversity of fern species

The Shannon-Wiener Species Diversity Index was used to determine fern species diversity (Nolan & Callahan, 2006). The Shannon-Wiener Species Diversity Index was calculated by adding the number of each species, the proportion of each species to the total number of individuals, and then multiplying the proportion by the natural log of the proportion for each species. This calculation for each species is then summed (1). The greater the number, the greater the species diversity. In an ideal situation, one would compare populations with the same number of individuals. The formula is:

$$H' = -\sum_{j=1}^{S} p_i \, In \, p_i \qquad (1)$$

where H' is the species diversity index, S is the number of species, and pi is the proportion of individuals of each species belonging to its species of the total number of individuals.

The Pielou Evenness Index was used to quantify both diversity and species richness (Zhang et al., 2012). While species richness refers to the number of different species present in a given area, evenness refers to the individual count of each species present (2). The equality calculation values of Pielou range from 0 (no equality) to 1 (complete equality). The formula is:

$$J' = \frac{H'}{In(S)} \qquad (2)$$

where J' = the evenness index, H' = the Shannon-Wiener index and S = the total number of species.

Measurement of relative chlorophyll content using SPAD-502 meter

A chlorophyll meter (SPAD-502) was used to determine the relative chlorophyll content. The chlorophyll content was measured by reading optical density (OD) on a spectrophotometer (Su et al., 2010). Samples were cleaned from soil with wash under running water. The sample was then inserted into the measuring head SPAD-502 meters and the measuring head is closed. The measurements with the SPAD-502 meter have produced relative SPAD meter values that are proportional to the amount of chlorophyll present in the leaf. The readings were recorded, and the process was repeated using samples of other fern species.

Statistical analysis

All the values of the Shannon-Wiener Index and Pielou Evenness Index and also Analysis of Variance (ANOVA) test was calculated. The ANOVA statistical test was done using SPSS Version 27. The Shannon-Wiener Index and Pielou Evenness Index was used to determine the species diversity of fern species. One-way analysis of variance (ANOVA) test was used to determine whether the observed results are significant (Bobbitt, 2021) and Microsoft Excel used to construct the graph.

Result and Discussion

Collections of fern samples

During the collection of fern species using a non-random sampling method in various areas of the forest, specific checkpoints (2, 3, 5, and 7) were targeted. Non-random sampling entails deliberately selecting areas based on certain requirements, such as vegetation types or ecological features. Fern specimens were identified and collected in a systematic manner at each designated checkpoint over the course of two days. This method enables a focused investigation of fern diversity in specific areas, yielding valuable insights into the distribution and composition of fern species in the designated areas of Hutan UiTM Cawangan Negeri Sembilan. Table 1 shows the list of disovered ferns. Based on the features of the collected samples, 8 families of ferns that represent 8 genera and 9 species being found. In checkpoint 2, there were 5 species being found, consisting of *Lygodium japonicum, Dicranopteris linearis, Taenitis blechnoides, Thelyteris oppositipinna* and *Selaginella microphylla*. In checkpoints 3 and 5, there is only 1 species found which are *Osmunda vachellii* and *Lycopodiella cernua*. At checkpoint 7, there were 2 species being found which are *Leptochilus pteropus* and *Selaginella willdenowii.* The highest species that was found between all checkpoints is the species found at the seventh checkpoint which is *Selaginella willdenowii* which have 52 individuals and the lowest species that was found between all checkpoints is the species found at the fifth checkpoint which is *Lycopodiella cernua* which have only 3 individuals.

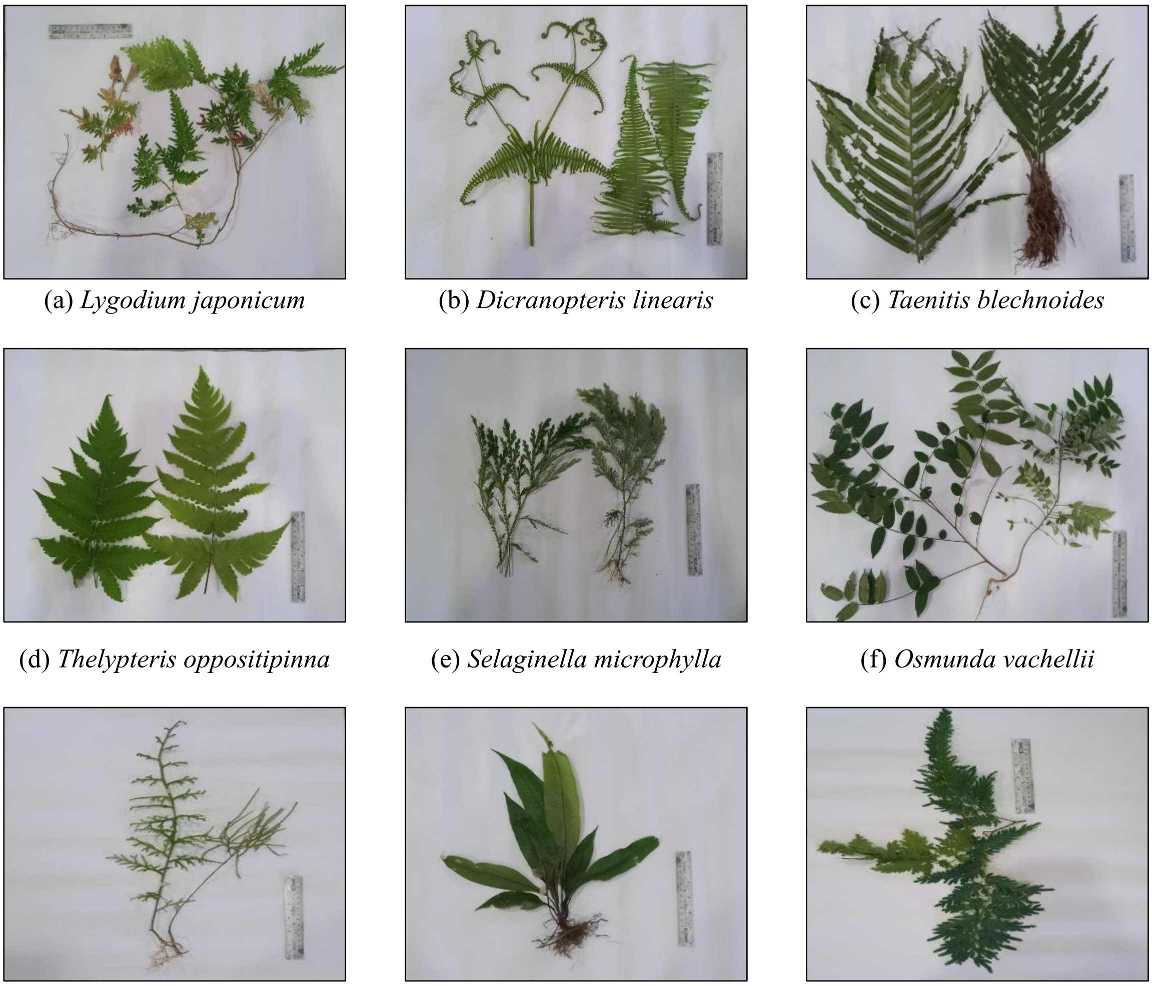
Table 1**.** Collected species of ferns in the Hutan UiTM Cawangan Negeri Sembilan

Checkpoint	No.	Family	Genus	Expected Species	Number of individuals
Checkpoint 2	1	Lygodiaceae	*Lygodium*	*Lygodium japonicum*	39
	2	Gleicheniaceae	*Dicranopteris*	*Dicranopteris linearis*	33
	3	Pteridaceae	*Taenitis*	*Taenitis blechnoides*	8
	4	Thelypteridaceae	*Thelypteris*	*Thelypteris oppositipinna*	14
	5	Selaginellaceae	*Selaginella*	*Selaginella microphylla*	45
Checkpoint 3	6	Osmundaceae	*Osmunda*	*Osmunda vachellii*	11
Checkpoint 5	7	Lycopodiaceae	*Lycopodium*	*Lycopodiella cernua*	3
Checkpoint 7	8	Polypodiceae	*Leptochilus*	*Leptochilus pteropus*	26
	9	Selaginellaceae	*Selaginella*	*Selaginella willdenowii*	52

Each species has been identified by its morphology and characteristics. Table 2 shows the morphology of each species. The characters and structure of the fern species are in accordance with the literature and images in reference (Xu & Deng, 2017) and website. The fern samples found were shown in Figure 3 (a-i). All images of fern samples were taken using a camera.

Table 2. Morphology of fern species

No.	Species	Morphology
1	*Lygodium japonicum*	• The fronds are long, lacy and finely divided along the wiry vines. • Rhizomes are 1-3 cm below the soil surface. • As in (Bradley, 2022).
2	*Dicranopteris linearis*	• Bipinnate fronds, main rachis divided into two distinct rachis and further divided into two branched of leafy branches. • Yellow single-pointed sori like shape around each subleaflets of frond along its vein. • As in (Yang et al., 2021b).
3	*Taenitis blechnoides*	• Simple pinnate frond, fertile fronds are narrower than the sterile fronds, sori linear and forming a narrow longitudinal band between midrib and margin. • Long rhizomes about 4-5 mm in diameter, with dark brown to black stiff bristles. • As in (Vaganov, 2021).
4	*Thelypteris oppositipinna*	• Simple pinnate, circular sori near the base of pinna towards apex. • Sporangia lacking glands or hairs distally but bearing a large spherical red gland at the end of a hair on sporangium-stalk. • As in (Sciandrello et al., 2021).
5	*Selaginella microphylla*	• Simple, scale-like leaves (microphylls) on branching stems from which roots also arise. • The stems are aerial, horizontally creeping on the substratum. • As in (Fang et al., 2021).
6	*Osmunda vachellii*	• Pinnate-pinnatifid leaves and it has fertile pinnae at the lower part of its frond. • Fertile fronds generate clusters of greenish sporangia that eventually turn brown or black. • As in (Trinh, 2018).
7	*Lycopodiella cernua*	• Extensive branching, horizontal stems that arch across the ground. • Erect branches are formed between rooting points and have sterile, spirally arranged, incurved and linear leaves that are all the same size and shape. • As in (Ollgaard, 1975).
8	*Leptochilus pteropus*	• Dark brown or dark green roots. • Have leaves that are medium to deep green with a leathery texture and short stalks. • As in (Miyoshi et al., 2019).
9	*Selaginella willdenowii*	• Iridescent fronds are mostly blue-green with pinkish hues depending on the angle. • They are composed of two leaf types, lateral and median leaves. • As in (Thomas et al., 2010).



(a) *Lygodium japonicum* (b) *Dicranopteris linearis* (c) *Taenitis blechnoides*

(d) *Thelypteris oppositipinna* (e) *Selaginella microphylla* (f) *Osmunda vachellii*

(g) *Lycopodiella cernua* (h) *Leptochilus Pteropus* (i) *Selaginella willdenowii*

Note: Checkpoint 2 (2.797742,102.222030) (a-e); Checkpoint 3 (2.795618, 102.224311) (f); Checkpoint 5 (2.796821, 102.223225) (g); Checkpoint 7 (2.795542, 102.224429) (h-i)

Figure 3. The images of fern species samples

Relative chlorophyll content

The relative chlorophyll content is an important measure that represents the stress and health of vegetation (Wu et al., 2023). It is one of the critical elements that determine crop growth and output (Kasim et al., 2018). The relative SPAD meter readings produced by measurements with the SPAD-502 meter are proportional to the amount of chlorophyll contained in the leaf. Table 3 shows the mean of relative chlorophyll content for each species. The range of relative chlorophyll content values for each species is 10.0 to 55.0. The highest average of relative chlorophyll content recorded is 52.07 ± 2.18^{a} which is *Osmunda vachellii* and the lowest is 10.63 ± 2.89^{f} which is *Dicranopteris linearis*. The percentage difference of soil pH between *Osmunda vachellii* and *Dicranopteris linearis* is 132.19%. The values (mean±SD) followed by dissimilar letters in each row in Table 3 are significantly different at $p \leq 0.05$ (ANOVA, Tukey's HSD post hoc test).

The high chlorophyll content observed in *Osmunda vachellii* (Figure 4) can be related to several factors such as photosynthetic efficiency, moisture retention and shading tolerance. Chlorophyll, the pigment responsible for capturing light energy, has become abundant in this fern species to maximize its photosynthetic efficiency (Zhang et al., 2014). This enables them to convert sunlight into chemical energy efficiently, allowing them to grow and survive in a variety of environments. *Osmunda vachellii* also requires a consistently moist environment to survive (Grimm et al., 2014). The high chlorophyll content aids in optimizing water use during photosynthesis indicating that chlorophyll-rich ferns have a mechanism for effectively balancing the need for carbon dioxide uptake with the need to minimize water loss through transpiration. Ferns can achieve the necessary energy conversion with less water loss by optimizing water use during photosynthesis. This is especially important in situations of excessive sunlight exposure, where the risk of excessive transpiration and water loss is higher (Zhang et al., 2014). Thus, ferns with more chlorophyll can capture and use light energy more efficiently, reducing the amount of water lost due to overexposure to sunlight. Apart from that, this fern species is also grown without additional nitrogen sources because of a symbiotic relationship with organisms capable of providing the necessary nitrogen. Nitrogen-fixing bacteria or mycorrhizal fungi are most likely involved in this symbiosis (Mus et al., 2016). Plants are able to get nitrogen from the soil, organic matter, or atmospheric nitrogen in various ecosystems. Some plants, such as ferns, have evolved mechanisms to form mutualistic relationships with nitrogen-fixing organisms. This helps the fern to absorb the nitrogen it requires for growth and development without the need for external nitrogen supplementation (Goyal & Habtewold, 2023). Because of this, its chlorophyll levels are consistently high. This demonstrates the critical role of symbiosis in providing nitrogen to ferns that cannot be substituted by mineral molecules.

Table 3. Relative chlorophyll content for each species

No.	**Species**	**Relative chlorophyll content**
1	*Lygodium japonicum*	20.07 ± 5.35^{def}
2	*Dicranopteris linearis*	10.63 ± 2.89^{f}
3	*Taenitis blechnoides*	38.27 ± 4.29^{bc}
4	*Thelyteris oppositipinna*	35.10 ± 1.56^{c}
5	*Selaginella microphylla*	16.50 ± 4.97^{ef}
6	*Osmunda vachellii*	52.07 ± 2.18^{a}
7	*Lycopodiella cernua*	50.23 ± 3.19^{ab}
8	*Leptochilus pteropus*	30.17 ± 4.09^{cd}
9	*Selaginella willdenowii*	28.63 ± 1.11^{cde}

Compared to other species that have low chlorophyll content, ferns can be influenced by several factors, including genetic variations, environmental conditions, and evolutionary adaptations. Ferns grown in low-light environments, such as dense forests or shaded areas, may have lower chlorophyll content (Pietrak et al., 2022). This is because chlorophyll is required for capturing light energy for photosynthesis, and ferns can reduce chlorophyll production in low light conditions. Furthermore, by lowering their chlorophyll content, some fern species have thrived in low light conditions. This adaptation allows them to make efficient use of limited light and survive in shaded areas. According to the research (Huang et al., 2022), most studies have shown that nutrient availability such as nitrogen and phosphorus, stress conditions, environmental conditions and light availability are the main factors that affect the concentration of chlorophyll. While chlorophyll is the primary pigment responsible for capturing light energy during photosynthesis in plants, including ferns, other pigments also play important roles. Ferns contain accessory pigments in addition to chlorophyll, which broaden the range of light wavelengths that can be absorbed for photosynthesis. Carotenoids, for example, are the pigments that give plants their yellow, orange, and red colours. Carotenoids act as accessory pigments in ferns, absorbing light in the blue and green regions of the spectrum (Swapnil et al., 2021). They help to maximize photosynthesis efficiency by transferring absorbed energy to chlorophyll.

Following that, xanthophylls are a type of carotenoids that contribute to the yellow coloration of leaves. Xanthophylls, like other carotenoids, help to capture light energy and transfer it to chlorophyll (Khoo et al., 2011). Aside from that, anthocyanins are a type of pigment found in some ferns that are not directly involved in light absorption for photosynthesis. Anthocyanins are the pigments that give plants their red, purple, and blue colours. These pigments may have protective properties, such as resistance to UV radiation, herbivores, and pathogens (Mattioli et al., 2020). Finally, phycobilins are commonly found in red algae and cyanobacteria, but some ferns may also contain phycobilins. Phycobilins absorb light in the blue and green spectrums, complementing chlorophyll's absorption spectrum. These pigments are essential in low-light environments because they improve light absorption and contribute to overall photosynthetic efficiency (Dagnino-Leone et al., 2022). As a result of the presence of these accessory pigments, ferns can absorb a wider range of light wavelengths than chlorophyll alone. This adaptation is especially useful in environments with varying light conditions, allowing ferns to thrive in a variety of ecological niches. Furthermore, the interaction of different pigments protects the plant from excessive light exposure and optimizes energy absorption for photosynthesis.

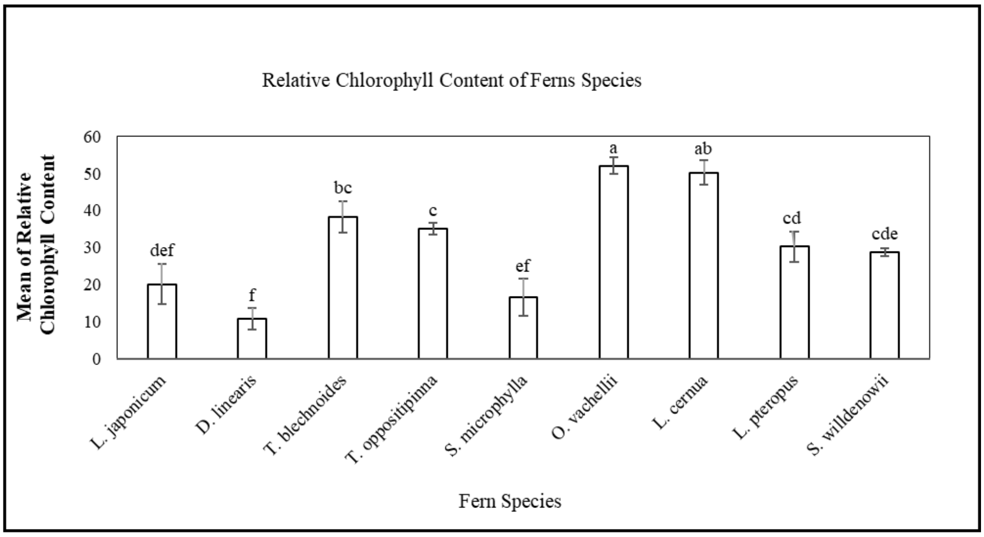


Figure 4. Chart of relative chlorophyll content

Species diversity of fern species

The Shannon-Wiener Species Diversity Index has been used to determine fern species diversity. The Shannon-Wiener Index (H) values indicate that species diversity involves two main components, which are species richness and evenness. They also found that Shannon diversity indexes normally vary from 1.5 to 3.5, with 4.5 considered quite rare. This study gained a value of 1.95 as shown in Table 4.

Table 4. The Shannon-Wiener Species Diversity Index

No.	Species	Number of individuals	p_i	In(p_i)	p_i x In(p_i)
1	*Lygodium japonicum*	39	0.17	-1.77	-0.30
2	*Dicranopteris linearis*	33	0.14	-1.97	-0.28
3	*Taenitis blechnoides*	8	0.03	-3.51	-0.11
4	*Thelyteris oppositipinna*	14	0.06	-2.81	-0.17
5	*Selaginella microphylla*	45	0.20	-1.61	-0.32
6	*Osmunda vachellii*	11	0.05	-3.00	-0.15
7	*Lycopodiella cernua*	3	0.01	-4.61	-0.05
8	*Leptochilus pteropus*	26	0.11	-2.21	-0.24
9	*Selaginella willdenowii*	51	0.22	-1.51	-0.33
Total	S = 9	230			H' = 1.95

The higher the value of H, the higher diversity of species in a particular community (Verberk, 2011). The lower the value of H, the lower the diversity. In this study, a Shannon-Wiener Index of 1.95 suggests a moderate to high level of species diversity in the community. The Pielou Evenness Index has been used to quantify both diversity and species richness. The Pielou Evenness Index is calculated by dividing the Shannon Diversity Index by the natural log of the richness.

$$\text{The Pielou Evenness: } J' = \frac{1.95}{In(9)} = 0.89 \quad (3)$$

In this study, the Pielou Evenness Index for this community is 0.89 as seen in (3). The Pielou index value is defined between 0 and 1 with 1 representing a community with perfect evenness. The index value decreases towards zero as the relative abundances of the species diverge from evenness (Jost, 2010). Species Evenness is highest when all species in a sample have the same abundance. A value of 0.89 indicates a relatively balanced distribution of species abundance, implying that the species are represented more evenly than if the value was lower. These values indicate that the community has a relatively high species diversity (Shannon-Wiener Index = 1.95), as well as an even distribution of abundance among species (evenness = 0.89). Therefore, these two indices indicate a healthy, balanced ecosystem in which different species coexist and share resources.

Species evenness in ecological communities refers to the relative abundance of different species present, whereas species richness refers to the total number of species. A community can have high species evenness but a low or high number of fern species (Kagaruki, 2021). When a community contains a large number of fern species and each species is relatively evenly distributed in terms of its abundance, high evenness can occur. This represents a well-balanced community in which no single species dominates the ecosystem. Several environmental factors, including stable conditions, low competition, and suitable habitat, may contribute to fern species richness and evenness (Yang et al., 2021a). Meanwhile, high evenness with a small number of fern species occurs when the community has a limited number of fern species, but each of these species has the same number of individuals. This could be because of ecological factors like niche partitioning or competitive exclusion, in which different fern species have adapted to occupy different ecological niches, resulting in similar population sizes for each species (Aros-Mualin, 2021). Evenness indicates that the few fern species present use available resources more evenly. Thus, the factors influencing species richness and evenness can differ depending on the community and ecological context.

Conclusion

The findings of the study indicate that fern composition has a relatively high species diversity. This study also suggests that each fern species collected has a different morphology and physiological performance that categorizes the species into specific classifications. The relative chlorophyll content in different areas of the fern depends on its morphology and physiological performance to survive. Overall, the results indicate that ferns have high biological significance that will benefit ecosystems. A few recommendations include increasing the time of collecting and the frequency of samples. Increasing the length of time for the collection of samples would result in more collected species and the discovery of new fern species. Aside from using journals, books, and websites to identify fern species or check their sporangium, there are other ways that may be used to make the results more dependable and trustworthy, such as using DNA barcodes. Furthermore, the variety and richness of ferns in this area are unknown. As a result, more research into the diversity of fern species in the Hutan UiTM Cawangan Negeri Sembilan is recommended in order to have advanced observations and discoveries in this region for the time being. The findings of this study could serve as a baseline for measuring biodiversity.

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Author Contribution

Nurhaziqa – conducted all the experimental work on fern species and contributed to discussion and paper writing; Lili Syahani – conceptualization, supervision on this work and contributed significantly to discussion and paper writing; Nor'aishah – review and editing.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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