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Characterisation of mucilage extract of Paku Midin (*Stenochlaena palustris*)

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Abstract—Plant mucilage has a wide range of uses in the food industry. The characterization of mucilage extract from *Stenochlaena palustris* (Paku Midin) especially was lacking, with limited studies focusing on the chemical composition, physical properties, and ecological implications of the plant. Therefore, understanding of the mucilage extract phytochemical content at different maturity stages and extracted plant parts, is crucial for its potential use as a functional food ingredient. The study was conducted to determine the chemical composition of mucilage extract from *S. palustris* through phytochemical and physicochemical analysis, besides, determining the amount of mucilage extract yield at different maturity. The study design has two treatments, namely mucilage from young and mature leaves. Mucilage extraction is carried out using aqueous extraction technique. This research offers greater focus on specific physicochemical properties namely pH, color, and water holding capacity. The phytochemical of plant mucilage was measured using instrumental approaches of UV-Vis highlighting on phytochemical components like flavonoid and phenolic compounds. The results indicate mucilage extract of young leaves is higher than mature leaves. Meanwhile, physicochemical analysis data suggest the acid nature of mucilage is due to the presence of uronic acid, water holding capacity of mucilage from young leaves is higher than mature leaves, and each samples have obvious color differences. Phytochemical analysis reveals total phenolic content for young leaf mucilage was higher at 0.041 mg GAE/g ± 0.003, while total flavonoid content was higher in mature leaf mucilage at 0.166 mg CEQ/g ± 0.002. Overall, this study not only explains clear relationship between leaf maturity and mucilage production, but further on characterizing the physicochemical and phytochemical properties of mucilage extract from *S. palustris* for two different treatments.

***Keywords*— *flavonoid; phenolic; phytochemical compounds; plant mucilage; S. palustris; UV-Vis*.**

INTRODUCTION

Polysaccharides, specifically hydrocolloids, are crucial molecules in determining the physicochemical and mechanical properties of food (Xu, 2017). Plant mucilage is one of the primary polysaccharides with various uses such as thickening, binding, strengthening, blending, and stabilising agents (Cakmak *et al.*, 2023). Examples of plants containing mucilage include *Aloe vera*, *Salvia hispanica* seeds, *Cordia dicotoma*, *Cactaceae*, *Abelmoschus esculentus*, *Trigonella foenum-graecum*, and *Moringa oleifera* (Tosif *et al.*, 2021). This mucilage can be obtained from various plant components such as seeds, bark, leaves, and the outer epidermal mucous layer of shoots (Mukherjee *et al.*, 2019). The potential health benefits of plant mucilage have led to its widespread use in pharmaceutical formulations, functional products, and dietary supplements.

S. palustris, or Paku Midin, is a fern species with significant potential as a functional food. This species, found in the tropical and subtropical regions of Asia, is rich in phytochemicals such as flavonoids and phenolics, which play important roles in preventing cardiovascular diseases, anti-ageing, and scavenging oxygen free radicals (Ndanusa *et al.*, 2020). With the increasing demand for functional foods and nutraceuticals, this study aligns with market trends showing rapid growth in the global health food market. While some previous studies have examined the chemical composition and physical properties of this species, comprehensive research on the total yield of *S. palustris* mucilage extract according to growth stages is still lacking.

A significant gap exists in understanding the chemical composition of S. palustris plant mucus, particularly the phenolic and flavonoid content, hindering the development of targeted applications and products. The proposed research aims to fill this gap by systematically investigating the phenolic and flavonoid content of S. palustris plant mucus using advanced analytical techniques, providing a comprehensive understanding of its chemical composition for potential applications in the food and pharmaceutical industries. Additionally, the exact values of phytochemical compounds in mucilage extracts at different maturation stages are unknown and require further investigation.

According to Royani *et al.* (2023) mucilage composition varies depending on plant species, part used, and extraction method. Understanding its chemical composition at different maturation stages is essential to unlock its potential health benefits and applications. A study by Xie *et al.* (2018) using samples of plants commonly found in aquatic areas demonstrated that mucus on stems, leaves, and young shoots decreases as leaves become mature. This explained that factors such as plant maturity, geographical location, and the plant part extracted can influence phytochemical content, highlighting the importance of considering these variables when analyzing the potential health benefits and applications of plant mucus.

This research is important as it aims to expand our knowledge of S. palustris plant mucus' chemical composition, which can lead to novel products and applications in the food and pharmaceutical industries. By exploring the potential health benefits and unique properties of this under-explored plant species, the study can contribute to innovation, growth, and sustainable utilization of S. palustris, while also promoting the development of more environmentally friendly and sustainable products. Therefore, characterising *S. palustris* mucilage extract is essential for its use as a functional food. The findings will not only advance knowledge of this unique fern species but also reveal its potential applications in health and the food industry.

MATERIALS AND METHODS

The sampling of the study material was conducted in the state of Sabah. The sampling location (Figure 1) was in Kampung Tengkurangoh situated in a small town called Tamparuli in the rural district of Tuaran. The main study material sampled from this location was *S. palustris*.

Fig. 1- Shows the habitat area of Paku Midin.

The chemicals used in this study were potassium sulfate (SYSTERM®), copper sulfate (R & M Chemicals), sulfuric acid (SYSTERM®), boric acid (SYSTERM®), hydrochloric acid (SYSTERM®), petroleum ether (Qrëc), sodium hydroxide (Qrëc), selenium tablets (Fisher Scientific), bromocresol green (Fisher Scientific), phenolphthalein (HANS), methanol (Labscan), isopropyl alcohol (Syarikat Jaya Kimia), Folin-Ciocalteu reagent (Merck), sodium carbonate (EMSURE®), gallic acid (Merck), sodium nitrate (EMSURE®), aluminum chloride (Bendosen), potassium sulfate (Merck).

A. Extraction of Paku Midin Leaves

Sample preparation is a crucial step in every scientific study, ensuring that the collected samples are properly handled and processed for advanced analysis. The sample preparation was carried out following the method by Yan *et al.* (2022) with some modifications.

Before mucus extraction, young and mature leaves picked on the same day were separated based on their physical appearance. The colour variation in *S. palustris* leaves indicates leaf maturity. Red leaves indicate young leaves, while green leaves indicate mature leaves (Pandiangan *et al.*, 2022). The collected leaves were carefully washed. A 100 g sample was heated in boiling water (80°C) for five minutes in a laboratory water bath (SciLab, Malaysia), then cooled in ice water. Excess water was removed, and the sample was cut into small pieces as suggested by Yan *et al.* (2022).

B. Extraction of Mucilage from Paku Midin Leaves

The mucus extraction method used is the water extraction technique. A 100 g sample was boiled in water at 80°C for 1 hour and 30 minutes in a laboratory water bath (SciLab, Malaysia), then immediately cooled in ice water. The sample was left in cold water (8°C) for 24 hours to extract the mucus. The liquid extract was separated and filtered using a layer of muslin cloth. The liquid extract was placed into centrifuge tubes and spun at 776 × *g* for 20 minutes to remove impurities using a centrifuge (Eppendorf, Germany). The supernatant was then collected, and isopropyl alcohol was added at a ratio of 2:3 of the filtrate volume to precipitate the mucus for extraction (Yan *et al.*, 2022).

The mucus was then filtered and dried using petri dishes until a constant weight was achieved at a temperature below 40°C. The hardened mucus was ground and sieved through sieve no. #22, then stored for further use. The experiment was conducted three times to obtain average values for both young and mature leaf samples. The mucus yield was calculated as follows:

$$\text{Mucilage yield (\%)} = \frac{\text{Dry mucilage weight(g)}}{\text{Raw leaves weight (g)}} \times 100\% \quad (1)$$

Chemical Analyses

Proximate analysis was conducted based on AOAC methods (2000). The proximate tests carried out included ash content, moisture content, protein content, fat content, crude fibre content, and carbohydrate content in the raw paku midin samples.

Physicochemical Analysis of Paku Midin Mucilage Extract

The pH determination analysis was tested using a pH meter (EUTECH Instruments, USA). 1.0 g of mucilage was weighed and dissolved in 1.0 ml of water to obtain a 1% w/v solution.

The colour profile analysis of the mucilage was tested using a colorimeter (Hunter Lab Colorflex, US). Three parameters were recorded: L*, a*, and b*. L* indicates the difference between light (L=100) and dark (L=0), a* represents the value between red (+a) and green (-a), and b* represents the difference between yellow (+b) and blue (-b) (Soares-da-Silva *et al.*, 2019).

Water Holding Capacity (WHC) was determined according to the method suggested by Sosulski *et al.* (1976) with slight modifications. 0.25 g of mucilage sample was weighed and placed into a centrifuge tube followed by 10 ml of distilled water. The tube was heated in a laboratory water bath (SciLab, Malaysia) at room temperature for 15 minutes with continuous shaking. Then, the centrifuge tube was spun at 3000 rpm for 15 minutes using a centrifuge (Eppendorf, Germany). After spinning, the supernatant was poured off and the tube with its contents was weighed again. The following formula was used to calculate the Water Holding Capacity:

$$\text{WHC (\%)} = \frac{(c)-(a+b)}{(b)} \text{x} 100\% \quad (2)$$

a = Weight of centrifuge tube (g)
b = Weight of dried sample (g)
c = Weight of centrifuge tube with sediment (g)

Analysis of Phytochemical Compounds in Paku Midin Mucilage Extract

The total phenolic content in the *S. palustris* mucilage sample was determined using the Folin-Ciocalteu (FC) reagent based on the method by Patle *et al.* (2020) with slight modifications. 0.1 g of *S. palustris* mucilage sample was placed into a beaker containing 10 mL of methanol and then subjected to ultrasonic extraction using an ultrasonic machine (Benson, US) for 15 minutes. Then, the sample was filtered, and 0.1 mL of the extract was mixed with 0.75 mL of twice-diluted FC reagent. The twice-diluted FC reagent was prepared by mixing 2.5 mL of stock solution with 2.5 mL of distilled water. The mixture was left at room temperature for five minutes, and then 0.75 mL of 7.5% Na_2CO_3 solution was added. The 7.5% Na_2CO_3 solution was prepared by dissolving 7.5 g of Na_2CO_3 powder in 100 mL of distilled water. The solution was stirred and left at room temperature for 1 hour. The absorbance of the solution was measured and read at 725 nm using a UV-Vis spectrophotometer. Standard solutions were prepared using gallic acid standard solution at concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL, r^2=0.998).

The total flavonoid content was determined colorimetrically based on the method by Bakar *et al.* (2009) with modifications according to Patle *et al.* (2020). 0.1 g of *S. palustris* mucilage sample was placed into a beaker containing 10 mL of methanol and then subjected to ultrasonic extraction using an ultrasonic machine (Benson, US) for 15 minutes. Then, the sample was filtered, and 0.5 ml of the extract was mixed with 2.25 ml of distilled water, followed by the addition of 0.15 ml of 5% NaNO2 solution. The 5% $NaNO_2$ solution was prepared by dissolving 2.5 g of the powder in 50 ml of distilled water. After six minutes, 0.3 ml of 10% $AlCl_3$ solution was added and left for five minutes. The $AlCl_3$ solution was prepared by dissolving 5.0 g of $AlCl_3$ powder in 50 ml of distilled water. Subsequently, 1.0 ml of NaOH solution was added, and the solution was thoroughly mixed using a vortex. The absorbance of the solution was measured and read at 510 nm with a UV-Vis spectrophotometer. Standard curves were prepared using catechin standard solution at concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml, $r^2 = 0.998$).

Statistical analysis

Data was analysed using SPSS Statistics version 29. The proximate analysis of the raw *S. palustris* samples was evaluated using one-way ANOVA. Conversely, the differences between the average extraction results, physicochemical, and phytochemical analyses will be evaluated using a free ANOVA test at a 95% confidence level with a value of ($p < 0.05$). All experiments were run three times in repetition, and the values are presented as mean ± standard deviation.

RESULTS AND DISCUSSIONS

Table 1 Analysis of proximate content of whole plant parts of *S. palustris*

Proximate analysis	Chemical content of raw *S. palustris* (%)
Moisture Content	32.21 ± 0.52
Ash Content	7.04 ± 0.10
Protein Content	15.55 ± 0.12
Fat Content	3.21 ± 0.20
Crude Fiber Content	13.57 ± 0.24
Carbohydrate Content	28.43 ± 0.53

Data presented as mean ± standard deviation (n=3).

From Table 1, moisture analysis indicates that *S. palustris* contains 32.21% ± 0.52 water, which is crucial for stability and potential applications in the food (Forsido *et al.*, 2021). The ash content, which refers to the inorganic residue remaining after combustion, is 7.04% ± 0.10, reflecting the mineral composition (Ismail, 2017). The protein content, determined by multiplying the total nitrogen by a specific protein factor, is 15.55% ± 0.12. High protein content is desirable as it affects emulsifying activity and ensures compliance with nutritional standards (Ganogpichayagrai & Suksaard, 2020; Fan *et al.*, 2022).

The fat content is 3.21% ± 0.20, important for assessing the lipid composition of *S. palustris*, which provides an energy source and serves as a carrier for fat-soluble vitamins (Field & Robinson, 2019). Fat content is also significant in the extraction of lipophilic bioactive compounds (Pan *et al.*, 2022). Crude fibre, measured at 13.57% ± 0.24, provides insight into the potential digestive health benefits (Dai & Chau, 2017). Lastly, the carbohydrate content is 28.43% ± 0.53, essential for evaluating the energy potential of *S. palustris* in the human diet and its role in regulating blood glucose and insulin metabolism (Julie *et al.*, 2023).

Table 2 Percentage yield and physicochemical analysis of the extract from *S. palustris*

	Sample type	
	Young leaves	Mature leaves
Percentage yield of mucilage extract	0.48^a ± 0.07	0.04^b ± 0.03
pH	5.61^a ± 0.14	6.62^b ± 0.07

Water holding capacity		$35.55^{a} \pm 0.21$	$10.72^{b} \pm 0.14$
Color of the extract	*L	$13.02^{a} \pm 0.05$	$34.81^{b} \pm 0.13$
	*a	$7.33^{a} \pm 0.12$	$4.51^{b} \pm 0.04$
	*b	$11.02^{a} \pm 0.30$	$25.33^{b} \pm 0.10$
TPC (mg GAE/g)		$0.04^{a} \pm 0.003$	$0.027^{b} \pm 0.002$
TFC (mg CEQ/g)		$0.122^{a} \pm 0.001$	$0.166^{b} \pm 0.002$

Data in the form of mean ± standard deviation (n=3). Different letters in the same row indicate significant differences at ($p < 0.05$). *L value, indicating brightness, *a value, representing the red-green chromaticity, *b value, representing the yellow-blue chromaticity, TPC, Total Phenolic Content; TFC, Total Flavonoid Content.

Mucilage from *S. palustris* is important in the food industry for producing functional products. Based on Table II, the yield of mucilage extract from young *S. palustris* leaves is 0.48% ± 0.07, while mature leaves yield 0.04% ± 0.03. A two-tailed t-test indicates significant differences in mucilage production at different growth stages ($p<0.05$), with higher yields from young leaves. This is supported by the study conducted by Yan *et al.* (2022) using the water extraction technique. The experiment found a dynamic relationship between leaf maturity and mucilage production. This difference is attributed to physiological changes during plant growth. Añibarro-Ortega *et al.* (2019) note that mucilage in leaves and stems primarily stores water and nutrients. Young *S. palustris* leaves, being in the early growth stage, have a greater capacity for mucilage production due to their active involvement in growth processes. Mucilage's hygroscopic properties help maintain moisture levels during water scarcity (Tosif *et al.,* 2021), crucial for optimal growth in young leaves. Additionally, mucilage layers store nutrients, essential for the nutritional needs of young shoots during periods when soil nutrient uptake is challenging. This ensures a steady nutrient supply for rapid growth and metabolic demands. Consequently, the need for water and nutrient storage affects mucilage yield, differing between young and mature leaves. Studies, such as Xie *et al.* (2018), show that mucilage yield decreases as plants mature.

The pH of mucilage extract from young *S. palustris* leaves is 5.61 ± 0.14, while for mature leaves, it is 6.62 ± 0.07. A two-tailed t-test shows significant differences in pH values ($p<0.05$), with mucilage from young leaves being slightly more acidic than from mature leaves. pH measures hydrogen ion activity, with values below 7 indicating acidity (Khandpur, 2019). Both samples have pH values below 7 but near neutral. Variations in pH are linked to the chemical composition of mucilage at different growth stages. Tosif *et al.* (2021) note that mucilage is composed of carbohydrates like L-arabinose, D-xylose, D-galactose, L-rhamnose, and galacturonic acid. Polysaccharides in mucilage include neutral components (D-xylose, L-arabinose, D-galactose) and acidic components (L-rhamnose, D-galacturonic acid) (Kaur *et al.,* 2018). The acidic nature of mucilage suggests the presence of uronic acids (Oh & Kim, 2022). As further explained by Hassan *et al.* (2022), the study found that increased mucilage yield is associated with higher pH due to the separation of acidic groups like uronic acids. Therefore, measuring mucilage pH is crucial to determine its safety for digestive systems. The near-neutral pH of both samples suggests they are generally safe and suitable for various formulations.

Water-holding capacity (WHC) refers to a material's ability to retain water against gravity. The table shows the WHC of mucilage extract from young *S. palustris* leaves is 35.55 ± 0.21 (g water/g dry sample), while for mature leaves it is 10.72 ± 0.14 (g water/g dry sample). A two-tailed t-test indicates significant differences in WHC between young and mature leaves ($p<0.05$). The higher WHC in young leaves is attributed to the presence of hydroxyl groups in the mucilage structure, which, according to Ma *et al.* (2023), enhances the viscosity and texture of food. Whereas, lower WHC in mature leaves is linked to higher solubility, reducing gel-forming ability (Tosif *et al.,* 2021).

High WHC in mucilage can be exploited to enhance the texture and mouthfeel of food products. It also contributes to greater moisture retention, preventing food from drying out and ensuring prolonged freshness. Conversely, low WHC can lead to liquid loss during processing and changes in the final product's texture (Shen *et al.,* 2022). Therefore, a higher WHC is preferred for better gel consistency, which is beneficial for food applications.

Color parameters *L, *a, and *b provide a comprehensive view of the color characteristics of mucilage extract from *S. palustris.* The *L value, indicating brightness, is 13.02 ± 0.03 for young leaves and 34.81 ± 0.07 for mature leaves, with significant differences ($p<0.05$). This shows that mucilage from mature leaves appears visually brighter. The *a value, representing the red-green chromaticity, is 7.33 ± 0.07 for young leaves and 4.51 ± 0.02 for mature leaves, also significantly different ($p<0.05$). A decrease in the *a value indicates a shift from red to green tones, likely due to a reduction in anthocyanins, which are higher in young leaves and known for their red color and health benefits (Mattioli *et al.,* 2020).

The *b value, representing the yellow-blue chromaticity, is 11.02 ± 0.17 for young leaves and 25.33 ± 0.06 for mature leaves, with significant differences ($p<0.05$). This indicates a noticeable shift towards yellow in mature leaves. Analyzing the color parameters of mucilage provides insights into the specific compounds associated with these visual colors. Understanding these color characteristics enhances the potential of *S. palustris* mucilage as a natural food coloring source with health benefits.

The TPC values obtained from *S. palustri*s mucilage extract are 0.041 mg GAE/g ± 0.003 for young leaves and 0.027 mg GAE/g ± 0.002 for mature leaves. The t-test indicates significant differences in TPC at different growth stages ($p<0.05$), with higher TPC in young leaves. This can be attributed to plant physiology, environmental influences, and specific developmental characteristics. According to Royani *et al.* (2023), each plant part has varying phenolic content, explaining the TPC differences in *S. palustris* mucilage at different maturities.

Phenolic compounds are secondary metabolites synthesized by plants in response to environmental stress or developmental signals (Kołton *et al.,* 2022). Young leaves, in early growth stages, might experience higher physiological stress and cell activity, leading to increased

phenolic production. These compounds act as crucial defense mechanisms, protecting plants from oxidative stress, UV radiation, and potential pathogen attacks (Naikoo *et al.*, 2019). As antioxidants, phenolic compounds help neutralize harmful free radicals and protect cells from oxidative stress (Kruk *et al.*, 2022). There is a close relationship between consuming antioxidant-rich foods and preventing human diseases. Understanding these compounds is essential for optimizing their use as functional foods.

The TFC values obtained from S. palustris mucilage extract are 0.122 mg CEQ/g ± 0.001 for young leaves and 0.166 mg CEQ/g ± 0.002 for mature leaves. According to the t-test, TFC in young and mature leaves is significantly different ($p<0.05$), with higher TFC in mature leaves. Flavonoids, diverse secondary metabolites, are known for their antioxidant properties and varied biological activities. Higher TFC in mature leaves can be linked to plant responses to developmental signals and environmental stimuli (Shomali *et al.*, 2022). As plants mature, they undergo physiological changes, including cell wall structure and composition, leading to increased flavonoid synthesis as a protective mechanism.

Additionally, the specific composition of mucilage components contributes to TFC differences. Mature leaves contain flavonoids like quercetin and kaempferol derivatives, present in smaller quantities in younger tissues, resulting in higher overall TFC. Research by Chear *et al.* (2016) on mature *S. palustris* shoots indicates that they synthesize more complex flavonoid molecules, such as kaempferol 3-O-b-glucopyranoside, contributing to higher flavonoid content in mature leaf mucilage. Flavonoids enhance the nutritional profile and functionality of mucilage extracts. As potent antioxidants, they actively neutralize free radicals and protect against oxidative stress (Dash & Hashim, 2016). This is beneficial for formulating functional foods aimed at improving overall health and reducing the risk of chronic diseases. Ndanusa *et al.* (2020) state that flavonoids and phenolics are crucial for preventing cardiovascular diseases, anti-aging, and eliminating oxygen free radicals.

CONCLUSIONS

The extraction of mucilage from both young and mature *S. palustris* leaves has been analyzed, indicating that young leaves produce more mucilage, with young leaves being more practical for maximizing mucilage yield, crucial for food industry applications. The pH values of both mucilage samples are nearly neutral, but mucilage from young leaves is more acidic. Mucilage color differs between the two samples, which can be exploited as a natural food colorant with health benefits. Phytochemical analysis focuses on flavonoid and phenolic content. The TPC for mucilage from young leaves is higher, while the TFC is higher in mucilage from mature leaves. This indicates differences in the synthesis of bioactive compounds depending on leaf maturity, which has implications for potential health benefits. Differences in phytochemical composition can also aid in the development of more effective extraction processes based on desired applications in functional food products.

The study acknowledges the limitations of UV-Vis spectroscopy, which has a broad and overlapping absorption spectrum, making it difficult to distinguish between materials with similar spectra, especially in complex plant extracts. This technique is only useful for determining the amount of flavonoid content and phenolic compounds, but not for identifying individual compounds. Further analysis using mass spectrometry can help overcome these challenges by providing information on molecular weight, allowing for the differentiation and identification of flavonoid and phenolic compounds, such as tiliroside, kaempferol, quercetin, and rutin found in *S. palustris*.

Overall, this study elucidates the relationship between leaf maturity and mucilage yield. Characterizing the physicochemical and phytochemical properties of *S. palustris* mucilage at different leaf maturity levels provides new insights to fill existing gaps. These findings also open broader opportunities for the use of *S. palustris* mucilage in various applications, from nutraceuticals to the food industry.

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