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Pharmacognostic studies on *Stenochlaena palustris* (Burm. f) Bedd

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ABSTRACT

Stenoclaena palustris (Burm. f) Bedd. (Family: Blechnaceae) commonly known as 'Climbing fern' or 'Paku midin' in Malay is an evergreen tropical fern popularly used by the Malaysian community. Earlier reports on pharmacological activities on the plant include significant antioxidant, antibacterial and antifungal activities. In the present paper, we report some pharmacognostic studies of the leaves and fronds since there are no standardization parameters for this plant reported in the literature. The transverse section of the leaves revealed dorsiventral nature. Stomata are absent in the upper epidermis. Transverse section of the fronds revealed numerous vascular bundles scattered throughout the section starting from the cortex region. The powder microscopy of the leaves revealed fragments of epidermal cells, mesophyll, covering trichomes, calcium oxalate crystals, xylem vessels and phloem fibres. The preliminary phytochemical screening of different extracts revealed presence of alkaloids, steroids, terpenoids, flavonoids, tannins and phenolic compounds, carbohydrates, mucilages, proteins and amino acids respectively in the plant. The findings of the study can be useful in establishing pharmacognostic standards for the plant.

Keywords: *Stenoclaena palustris* (Burm. f) Bedd., Macroscopy**,** Microscopy, Physicochemical parameters, Preliminary phytochemical studies

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INTRODUCTION

Plants have been used as a source of vegetables and for medicinal purposes since time immemorial. Many plants contain flavonoids and polyphenols, as secondary metabolites that serve as a source of natural antioxidants. Thus, dietary antioxidants have attracted the attention of the researchers since they can protect the body from oxidative stress, which is regarded as prime cause of several deadly diseases including ageing, cardiovascular diseases and cancer. Adulteration in herbal samples has been the greatest challenge in ensuring their quality. Several countries have intensified their efforts in laying down standardization parameters for various traditional herbs. The World Health Organization has set up several guidelines for the standardization of herbs and recommends their application in all medicinal plants to ensure safety and efficacy.

Stenoclaena palustris (Burm. f) Bedd. (Family: Blechnaceae), commonly known as 'Climbing fern' or 'Paku midin' in Malay, is an evergreen tropical fern popularly used by the Malaysian community in their traditional vegetable salads 'Ulam'. The reddish young fronds are harvested from wild and sold in local markets as an edible wild vegetable**.** In folk medicines, the plant is used for treatment of fever and skin diseases, especially for the older people among the most ancient tribe 'Orang asli' [1, 2]. Earlier reports on pharmacological activities revealed significant antioxidant [3, 4], antibacterial [4], and antifungal [5] activities. There are also studies that represented *S.*

palustris can be used as natural food preservatives [6]. The plant is reported to contain steroids, flavonoids and alkaloids [1-3], Presence of phosphorus and potassium have been also identified in the plant [3]. There are no reports on the pharmacognostical parameters available in the literature. In the present paper, we report the macroscopical, microscopical and physiochemical parameters of *S. palustris* leaves and fronds. The studies were performed in accordance with the methodologies of WHO General Guidelines for Herbal Drug Standardization [7].

MATERIALS AND METHODS

Plant material

The fresh plant material was collected from the well grown and matured shrubs from Ipoh and authenticated. After authentication, the plant materials were collected in bulk. The fresh plant material was used for histological analysis. The remaining plant materials were shade dried, milled in to coarse powder and preserved for other studies.

Chemicals and reagents

All the chemicals, solvents and reagents used in the study were of standard analytical grade.

Macroscopy

Macroscopical studies were performed by carefully observing the plant parts by using a lens. The colour, odour, taste and texture were studied.

Microscopy

Thinnest possible transverse sections were taken from fresh leaves and young fronds separately and treated in chloral hydrate solution with gentle warming [8]. Few selected sections were stained with phloroglucinol and concentrated hydrochloric acid (1:1). After washing the sections with water, the stained sections were mounted on microscopic slide. The sample was covered with glycerin and a cover slip. The sections were then examined under binocular compound optical microscope (OLYMPUS; CX21FS1). The images were photo documented using a camera.

Powder microscopy

Powder microscopic characteristics of the leaves were studied by heating a small quantity the powder with small amount of chloral hydrate solution for 1-2 min [8]. Lignified tissues were confirmed after staining with a few drops of mixture of phloroglucinol and concentrated hydrochloric acid (1:1). In order to observe starch grains, small amounts of powder were mounted separately in N/20 iodine solution. The starch grains appeared light blue in colour. Detection of calcium oxalate crystals were carried out by treating the powdered sample with water followed by observation.

Physico-chemical analysis

Physico-chemical parameters included determination of moisture content, ash and extractive values. The parameters were studies according to the procedures laid down in British Pharmacopoeia [9]. The behaviour of the powder plant materials with different chemical reagents were studied according to the recommended method [10, 11]. The powders, after being treated with reagent were examined under visible light and UV at 366nm and 254nm.

Preliminary phytochemical studies

Preliminary phytochemical studies for the leaves and fronds were performed separately. A known quantity of dried plant material (10 g) was extracted successively with petroleum ether (40-60ºC), chloroform, methanol and distilled water. Following extraction, the liquid extracts subjected to fluorescent analysis to identify presence of any fluorescent phytoconstituents within them. The liquid extracts were then separately dried. The color, consistency and extractive values of all extract were noted. Presence of class of phytochemicals was determined by performing preliminary phytochemical studies [12-14].

RESULTS AND DISCUSSION

Macroscopy:

The macroscopic characteristics of the leaves and fronds were examined. The colour of the leaves was green with smooth texture but no characteristic odour and taste. The colour of the fronds was reddish with light green tint, smooth texture, no characteristic odour and taste.

Microscopy:
Transverse section of the leaves showed dorsiventral nature of the leaves (Fig. 1a). Upper and lower epidermis consists of wavy walled, compactly arranged cells covered by thin cuticle. There were few non lignified covering trichomes appear on both sides of the leaves. Numerous vascular bundles are seen at the midrib region. In the vascular bundles, the xylem vessels are surrounded by the phloem fibres. Collenchymatous tissues are observed at both the lower and upper portion of the midrib and give support to the midrib region.

Transverse sections of the fronds (Fig. 1b) revealed single layered and compactly arranged epidermis, below which collenchymatous tissues are observed. The pith is composed of parenchymatous cells. Numerous vascular bundles are seen scattered. The xylem vessels are surrounded by the phloem fibres.

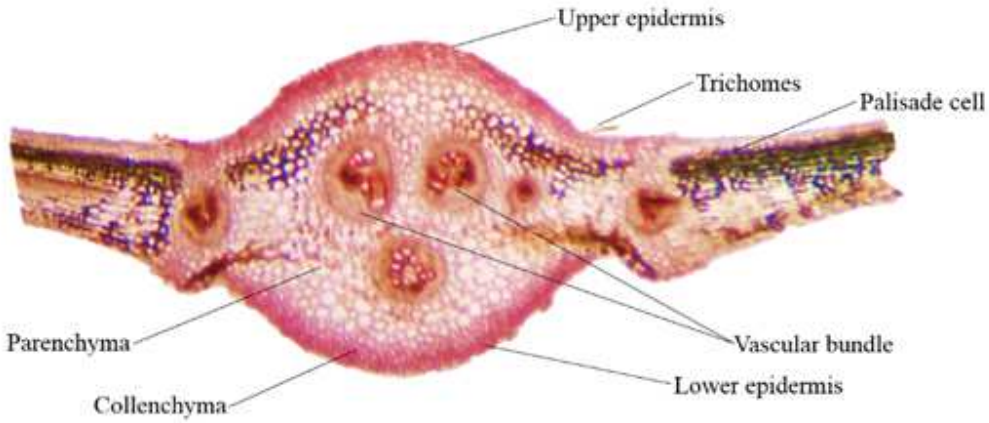


Fig. 1a: Transverse section of *S. palustris* leaf

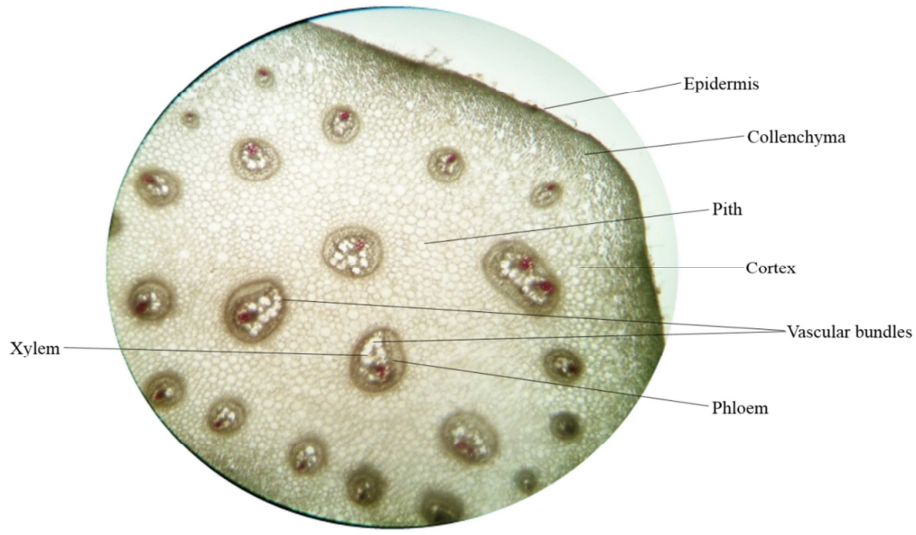
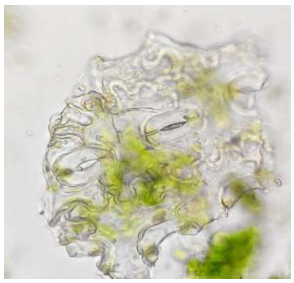


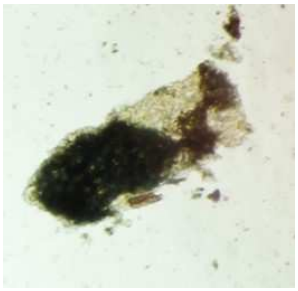
Fig. 1b: Transverse section of *S. palustris* frond

Powder microscopy
The results of the powder microscopy of the leaves are presented in Fig. 2. The powder microscopy of the leaves revealed fragments of epidermal cells, mesophyll, covering trichomes, calcium oxalate crystals, xylem vessels and phloem fibres.

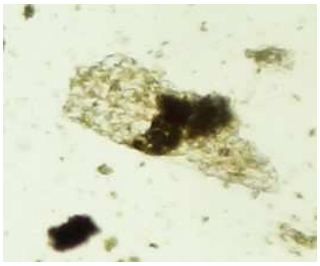
Upper epidermis (absence of stomata)



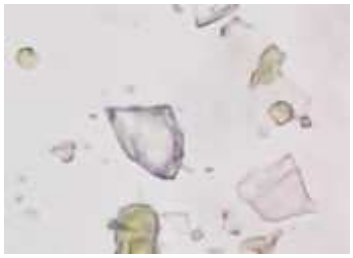
Lower epidermis (presence of stomata)



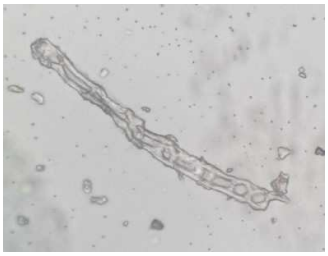
Mesophyll cell



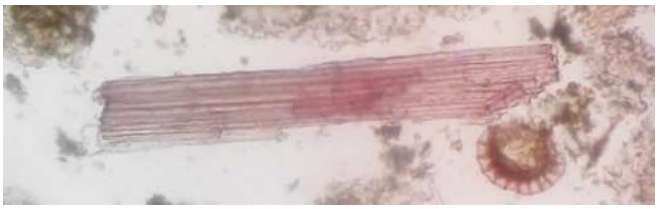
Epidermal cell



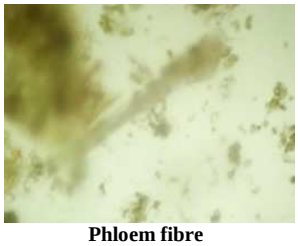
Calcium oxalate crystals



Trichome



Xylem vessel



Phloem fibre

Fig. 2: The powder microscopy of *S. palustris* leaves

Physicochemical analysis:

The percentage of total ash, acid-insoluble ash, water soluble ash, water soluble extractive, ethanol soluble extractive and moisture content are presented in Table 1. Behaviour of the powdered plant material with different chemical reagents was studied. The changes in colour the powdered samples were observed under visible light and UV at 366nm and 254nmand the results are presented in presented in Table 2.

Table 1: Physico-chemical analysis of *S. palustris*

Parameters	Value (% w/w)	
	Leaves	Fronds
Total ash	9.94 ± 0.35	9.19 ± 0.49
Acid-insoluble ash	3.09 ±0.38	3.60 ±0.41
Water-soluble ash	4.34 ±0.58	5.78 ±0.67
Ethanol soluble extractive	3.54 ±0.48	4.97 ±0.59
Water soluble extractive	15.85 ±1.53	14.70 ±1.78
Moisture content	16 ±2.56	11 ±1.58

Results expressed as Mean ±SD from three observations

Table 2: **Powder analysis of *S. palustris* at short UV, long UV and visible light**

Treatment	Plant part	Observation		
		Short UV	Long UV	Visible light
Powder alone	Leaf	Black	Black	Green
	Frond	Black	Black	Green
Powder + 50% H_2SO_4	Leaf	Dark green	Black	Green
	Frond	Dark green	Dark green	Brownish green
Powder + 50% HNO_3	Leaf	Dark green	Black	Orange
	Frond	Dark green	Black	Dark red
Powder + 50% HCl	Leaf	Black	Black	Green
	Frond	Green	Black	Brown
Powder + 5% KOH	Leaf	Dark green	Dark green	Brownish green
	Frond	Black	Black	Brownish green
Powder + 1N NaOH	Leaf	Dark green	Dark green	Brownish green
	Frond	Black	Black	Black
Powder + 5% $FeCl_3$	Leaf	Dark green	Black	Yellowish green
	Frond	Dark green	Black	Yellowish green
Powder + acetic acid	Leaf	Dark green	Black	Yellow
	Frond	Dark brown	Black	Coffee brown
Powder + N/10 Iodine	Leaf	Dark green	Black	Green
	Frond	Dark green	Black	Green
Powder + ammonia	Leaf	Dark green	Black	Green
	Frond	Dark green	Black	Green

Preliminary phytochemical studies

Preliminary screening of phytochemicals is a valuable step in the detection of the classes of phytochemicals present in crude drugs that may subsequently help in drug discovery process. The powdered leaves and fronds, after being extracted successively with different solvent were studied for colour, consistency, extractive values of the extracts. The liquid extracts were further observed under visible light and UV at 366 and 254 nm respectively (Table 3). Results of the preliminary phytochemical studies of different extracts are presented in Table 4.

Table 3: C**olour, consistency and extractive values of different extracts of *S. palustris* after successive extraction**

Extract	Plant part	Consistency	Yield (% w/w)	Colour		
				Visible light	UV	
					254 nm	366 nm
Petroleum Ether	Leaf	Greasy	0.23	Green	Light green	Orange
	Frond	Greasy	0.60	Green	Brown	Orange
Chloroform	Leaf	Greasy	0.71	Green	Dark green	Brick red
	Frond	Greasy	0.91	Green	Dark green	Brick red
Methanol	Leaf	Sticky	1.65	Green	Dark green	Brick red
	Frond	Sticky	2.41	Green	Dark green	Brick red
Distilled Water	Leaf	Sticky	2.59	Brown	Black	Green
	Frond	Sticky	3.93	Brown	Black	Green

Table 4: **Preliminary phytochemical studies of different extracts of *S. palustris***

Compounds	Leaf Extracts				Frond Extracts			
	Pet. Ether	Chloroform	Methanol	Aqueous	Pet Ether	Chloroform	Methanol	Aqueous
Steroids	+	+	-	-	+	+	-	-
Tannin and Phenolic Compounds	-	-	+	+	-	-	+	+
Flavonoids	-	-	+	+	-	-	+	+
Carbohydrates	-	-	-	+	-	-	-	+
Proteins and Amino acid	-	-	-	+	-	-	-	+
Alkaloids	-	+	+	-	-	+	+	-
Terpenoids	+	+	-	-	+	+	-	-
Gum and Mucilage	-	-	-	+	-	-	-	+

'+' = present; '-' = absent

CONCLUSION

S. palustris is well-known as an edible plant but has been widely used for its medicinal property in traditional system of medicine. In spite of several medicinal attributes of this plant by previous researchers, reports on the pharmacognostical studies are not available in the literature. The macroscopic and microscopic features may serve as important characteristics for identifying the plant. The physicochemical parameters can be helpful in detecting adulteration. The findings of the study can be useful in establishing pharmacognostic standards for the plant.

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REFERENCES

[1] TT Chai; MT Kwek; HC Ong; FCS Wong. *Food Chemistry*, **2015,** 186, 26-31.
[2] Adenan; S Eko. *Univerca Medicina*, **2010,** 29(3), 123-128.
[3] TT Chai; E Panirchellvum; HC Ong; FCS Wong. *Botanical Studies*, **2012,** 53, 439–446.
[4] Y Ponnusamy; NJY Chear; S Ramanathan; V Murugaiyah, CS Lai. *J Nat Prod Plant Resour*, **2013,** 3(6), 14-18.
[5] Z. Zuraini; S Sasidharan; KS Roopin; M Nithiyayini. *Pharmacologyonline*, **2010,** 1, 233-237.
[6] V Sumathy; LS Jothy; Z Zuraini;.S Sasidharan. , S. *Mal J Nutr*, **2010,** 16(3), 439-446.
[7] S. Shrikumar; MU Maheswari; A Suganthi; TK Ravi. WHO Guidelines for herbal drug Standardisation. Pharmainfo.net.,[cited 2004 September 19].Available from www.pharmainfo.net/reviews/whoguidelines-herbal-drug-standardization.
[8] WC Evans. Trease and Evans Pharmacognosy, 16th ed., London, W.B Saunders, **2009**; pp. 538.
[9] British Pharmacopoeia 2012. Vol. V, London: The Stationery Office, **2012**, pp. A282-309.
[10] M Kumar; P Mondal; S Borah; K Mahato. *Int J Pharm Pharm Sci*, **2013,** 5 (2), 307-309.
[11] J Deb; GK Dash. *Der Pharmacia Lettre*, **2014,** 6 (3), 61-66.
[12] Carmen R-OJd; Willam HMJ; Morales G;, Carmen AD; Nataly JG; Stefany COS, et al. *Res Pharm Sci* **2016,** 11(1), 15-22.
[13] KR Khandelwal. Practical pharmacognosy, Techniques and Experiments, 17th ed., Pune, Nirali Prakashan publisher, 2007; pp 149-154.
[14] S Lanjhiyana; D Garabadu; D Ahirwar; AC Rana; B. Ahirwar; SK Lanjhiyana. *Der Pharmacia Lettre*, **2011**, 3(1), 319-333.