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Pharmacognostic Studies On *Diplazium Esculentum* (Retz.) Sw.

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ABSTRACT

Diplazium esculentum (Retz.) Sw. (Family: Athyriaceae) (Figure 1), a common pteridophytes, is the most popular edible fern used by the Malaysian community in culinary and as a medicinal plant in the traditional system of medicine. Traditionally, the plant is used in treating headache, pain, fever, wounds, dysentery, glandular swellings, diarrhea, and various skin infections. Reported pharmacological and biological properties of this plant include laxative, anti-inflammatory, antioxidant, anthelmintic, antimicrobial, and cytotoxic activities. In the present paper, we report some pharmacognostic studies on the leaves since there are no standard parameters for this plant reported in the literature. The transverse section of the leaf showed presence of nonlignified covering trichomes, cuticle, upper and lower epidermis, palisade cells, xylem vessels, phloem fibers, parenchyma, collenchyma's and mesophyll. Powder microscopy showed the existence of anomocytic stomata and calcium oxalate crystals. Preliminary phytochemistry screening of different extracts showed presence of steroids, triterpenoids, tannins and

phenolic substances, flavonoids, carbohydrates, gum and mucillages. Finding can be useful in establishing pharmacognostic standards for the plant.

Key words: *Diplazium esculentum (Retz.) Sw., Macroscopy, Microscopy, Physicochemical analysis, Preliminary phytochemical studies*

INTRODUCTION

Plants have been used as the major source of medication since ancient times. Several reports have been presented by earlier researchers that the vegetables provide a good source of remedy against several diseases. Plants usually contain polyphenols and flavonoids as secondary metabolites that act 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.

Figure-1: *Diplazium esculentum* (Retz.) Sw.

Diplazium esculentum (Retz.) Sw. (Family: Athyriaceae) (Figure1), a common pteridophytes, is the most popular edible fern used by the Malaysian community in culinary and as a medicinal plant in the traditional system of medicine. The plant is mainly terrestrial, growing in humid lowland to high mountain forests and occasionally on limestone rocks [1].

Traditionally, the aerial parts are cooked and eaten by old people to maintain their health [2]. Aerial parts are also used to treat fever, dermatitis, and measles in ethnomedicine [3]. The leaves are believed to be a cure for headache, pain, fever, wounds, dysentery, glandular swellings, diarrhea, and various skin infections [4]. Boiled young fronds are eaten for laxative effect [5]. The plant is reported to contain steroids, triterpenoids, phenols, flavones, flavonoids such as myrcetin and alphatocopherol [2,6]. Pharmacological properties such as laxative [5], anti-inflammatory [7, 8], antioxidant [9], anthelmintic [10], antimicrobial [11], cytotoxic [11,12] activities have been reported.

MATERIALS AND METHODS

Plant material

The fresh leaves of *D. esculentum* Retz. were collected from the well grown and matured herbs at its natural habitat from Ulu Kuang, Perak and authenticated. The fresh leaves were used for histological studies. The remaining leaves were shade dried, milled in to coarse powder and preserved for other studies.

Chemicals

The chemicals, solvents and reagents used in the study will be of standard analytical grade.

Macroscopy

Morphological studies were performed by carefully observing the plant parts under day light using a convex lens. The color, odour, taste and texture were examined.

Microscopy

Few fresh leaves were boiled with chloral hydrate solution for 20 min. Thinnest possible transverse sections of the leaves was taken and stained with phluoroglucinol and hydrochloric acid in ratio 1:1. The selected sections were mounted on microscopic slides. The samples were covered with glycerin and a cover slip and examined under binocular optical compound microscope (Model: CX21, Serial no.: 8A08844). The images were photo documented using camera. The fresh samples were separately stained with 1% safranin solution, N/20 iodine solution and distilled water to observe mucillage, starch granules and calcium oxalate crystals respectively [13-15].

Powder microscopy

The powder microscopy characteristics of the leaves were studied by staining the coarse powder separately with phluoroglucinol-hydrochloric acid (1:1), N/20 iodine solution and distilled water to identify the presence of lignified tissues, starch grains and calcium oxalate crystals respectively [16].

Physico-chemical analysis

Physico-chemical analysis of the dried leaves included determination of moisture content, ash and extractive values. The parameters were studied according to the procedures laid down in British Pharmacopoeia [17]. The behaviour of the powder plant materials with different chemical reagents were studied according to the recommended method [18]. The powders, after being treated with reagent were examined under visible light and UV at 366nm and 254nm.

Preliminary phytochemical studies

About 10 g of the dried plant materials were extracted successively by ultrasonic extraction using petroleum ether (40 - 60˚C), chloroform, methanol and distilled water [19, 20]. The liquid extracts were subjected to fluorescence analysis to identify the presence of any fluorescence compound within them [21]. Then the extracts were separately dried [22]. The colour, consistency and extractive values of all extracts were determined. The presences of various classes of phytoconstituents were confirmed by performing recommended phytochemical tests on the extracts.

RESULTS AND DISCUSSION

Macroscopy

The colour of the leaves was green with no characteristic odour and taste. The fracture was smooth.

Microscopy

The transverse section of the leaves (Figure 2) showed upper and lower epidermis covered with thin cuticle. The epidermal layer consists of wavy walled and compactly arranged cells. Below the upper epidermis, there are few layers of elongated, compactly arranged palisade cells. The There were few non-lignified covering trichomes appear on both sides of the leaves. The vascular bundle consisted of xylem vessels surrounded by phloem fibres. Collenchymatous tissues are observed at both the lower and upper portion of the midrib and give support to the midrib region.

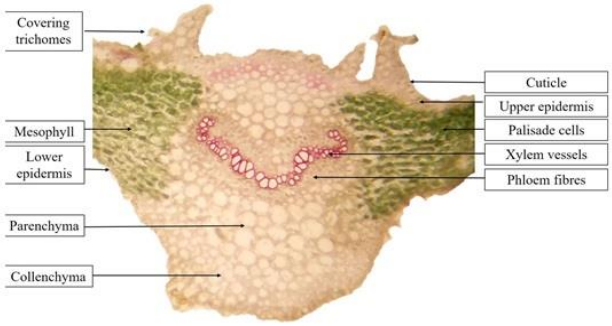

Figure-2: Transverse section of the leaf of D. esculentum (10x magnification)

Powder microscopy

The results of powder microscopy were shown as in Figure 3. The powder microscopical characteristics revealed fragments of mesophyll, uniseriate multicellular covering trichomes, calcium oxalate crystals, anomocytic stomata, xylem vessels and phloem fibres. Starch grains are absent.

Physico-chemical 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 observed under visible light and UV at 254 nm and 366 nm are presented in presented in Table 2.

Figure-3: Powder microscopy of *D. esculentum* leaves (10x magnification)

Table 1: Physicochemical analysis of *D. esculentum*

Parameters	**Value (%)**
Total ash	8.1 ± 0.76
Acid insoluble ash	3.05 ± 0.34
Water soluble ash	3.10 ± 0.27
Water soluble extractive	18.2 ± 0.86
Ethanol soluble extractive	3.9 ± 0.34
Loss on Drying	10.8 ± 0.72
Results expressed as Mean ± SD from three observations	

Table 2: Behaviour of the *D. esculentum* leaf powder with different chemical reagents

Treatment	Leaves		
	Visible	254 nm	366 nm
Powder + Distilled water	Green	Dark green	Black
Powder + 5% $FeCl_3$	Green	Dark green	Black
Powder + Glacial Acetic acid	Light brown	Green	Green
Powder + 5% KOH	Brownish green	Dark green	Dark green
Powder + 5% NaOH	Green	Dark green	Dark green
Powder + Conc. HCl	Green	Dark green	Dark green
Powder + Conc. H_2SO_4	Dark brown	Green	Dark green
Powder + Conc. HNO_3	Orange	Green	Dark green
Powder + N/10 Iodine	Green	Dark green	Dark green
Powder + Ammonia	Green	Dark green	Dark green

Preliminary phytochemical studies

The powdered leaves, 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). Preliminary phytochemical screening was performed on different extracts and the results are presented in Table 4.

Table 3: Ccolour, consistency, and extractive values of various extracts of *D. esculentum*

Extract	Consistency	Yield (%w/w)	Observation		
			Day light	UV 254 nm	UV 366 nm
Pet. ether	Greasy	0.27%	Olive green	Olive green	Brick red
Chloroform	Greasy	1.02%	Dark green	Black	Deep red
Methanol	Sticky	3.66%	Dark green	Dark green	Deep orange
Water	Crystalline	5.07%	Brown	Green	Blue black

Table 4: Preliminary phytochemical analysis of various extracts of *D. esculentum*

Test for	Extracts			
	Pet. ether	Chloroform	Methanol	Aqueous
Alkaloid	-	-	-	-
Carbohydrates	-	-	-	+
Flavonoids	-	-	+	+
Gums and mucilages	-	-	-	+

Protein and amino acid	-	-	-	+
Steroid and sterols	+	+	-	-
Tannins	-	-	+	+
Terpenoids	+	+	-	-
'+' = present; '-'= absent				

CONCLUSION

It is well known that *D. esculentum* is widely used as an important ingredient in the traditional Malaysian salad '*Ulam*' or cooked and eaten by the people to maintain their health. The plant is highly valued in traditional medicinal practice which reflects from its wide application in treatment of various ailments. Many pharmacological studies also have been carried out by several researches and documented significant activities. But until now, there is no pharmacognostical studies found in the literature. The studies and findings in this work may be helpful to the future investigators in the process of its identification and subsequently useful in establishing pharmacognostic standards for the plant.

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REFERENCES

1. Wei, R., Toward a new circumscription of the twinsorus-fern genus *Diplazium* (Athyriaceae): A molecular phylogeny with morphological implications and infrageneric taxonomy. Taxon, **2013.** 62(3), p. 441-457.
2. Kaushik, A., et al., FRAP (Ferric reducing ability of plasma) assay and effect of *Diplazium esculentum* (Retz) Sw. (a green vegetable of North India) on central nervous system. Indian J. Nat. Prod. Resour, **2012.** 3(2), p. 228-231.
3. Roosita, K., Medicinal plants used by the villagers of a Sundanese community in West Java, Indonesia, J Ethnopharmacol, **2008.** 115(1), p. 72-81.
4. Akter, S., et al. Investigation of In vitro Antioxidant, Antimocrobial and Cytotoxic activity of *Diplazium esculentum* (Retz). SW, *Int J Adv Pharm Biol Chem*, **2014.** 3(3), p. 723-733.
5. Kagyung, R., et al., Ethnomedicinal plant used for gastro-intestinal disease by Adi tribes of Dehang-Debang Biosphere Reserve in Arunachal Pradesh. Indian J. Trad. Knowledge, **2010.** 9(3), p. 496-501.
6. Upreti, K., et al., Ethnomedicinal uses of pteridophytes of Kumaun Himalaya, Uttarakhand, *India J Am Sci*, **2009.** 5(4), p. 167-170.
7. Kaushik, A., et al., Preliminary studies on anti-inflammatory activities of *Diplazium esculentum* in experimental animal models, *Int J Pharm Sci Res*, **2011.** 2(5), p. 1251-1253.

8. Ravikiran, G., et al., An annual review on anti-inflammatory medicinal plants. *Int J Phytother*, **2012.** 2(1): p. 23-27.
9. Rahmat, A., Determination of total antioxidant activity in three types of local vegetables shoots and the cytotoxic effect of their ethanolic extracts against different cancer cell lines, *Asia Pac J Clin Nutr*, **2003.** 12 (3): p. 308-311.
10. Amit, S. and Singh, FM., *In-Vitro* Anthelmintic activity of *Diplazium esculentum* (Retz.) Swiss rhizome extract, *J Pharmacog Phytochem*, 2012.14: p. 84-87.
11. Ullah, MO., et al., Antibacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. *Asian Pac J Trop Biomed*, **2013.** 3(1): p. 1-7.
12. Akter, S., Investigation of *in vitro a*ntioxidant, antimicrobial and cytotoxic activity of *Diplazium esculentum* (Retz), Sw, *Int J Adv Pharm Bio Chem*, **2014.** 3(3): p. 723-733.
13. Wallis, TE., Text book of Pharmacongosy, CBS Publishers and Distributors, Delhi, **1985.** 571.
14. Prabhu, K., et al., Pharmacognostic investigation of the leaves and stems of *Viburnum erubescens* Wall. ex DC. *Trop J Pharm Res*, **2009.** 8(6): p. 557-566.
15. Abere, TA., and Onwukaeme DN, Pharmacognostic evaluation of the leaves of *Secamone afzelii* (Schult) K Schum (Asclepiadaceae). *Trop J Pharm Res*, **2012.** 11(1), 125-131.
16. Evans, WC., Trease and Evans Pharmacognosy, WB Saunders, London. **2009.**
17. British Pharmacopoeia 2012. Vol. V, The Stationery Office, London, **2012.**
18. Dash, GK., Studies on *Tragia involucrata* Linn. *Aryavaidyan*, **2002.**16(4): p. 226-233.
19. Harborne, JB., Phytochemical Methods - A Guide to Modern Techniques of plant analysis, Chapman and Hall, London, **1998.**
20. Mahitha, B., et al., *In vitro* antioxidant and pharmacognostic studies of leaf extracts of *Cajanus cajan* (L.) Millsp, *Indian J Pharm Sci*, **2015.** 77(2): p. 170-177.
21. Anupama, N., and Madhumitha, G., Pharmacognostic Standardization, and Anti-microbial studies of dried Carissa carandas fruits. *Int J PharmTech Res*, **2015.** 8(8): p. 206-210.
22. Gupta, PC., et al., Pharmacognostic studies of the leaves and stem of *Careya arborea* Roxb, *Asian Pac J Trop Biomed*, **2012.** 2(5): p. 404-408.