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A comparative and biochemical evaluations of Edible Parts of *Diplazium esculentum* (Retz.) SW. a Locally available Fern of Assam at Different Growing Stages

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ABSTRACT

Edible portion of *Diplazium esculentum* (Retz.) sw. at four different growing stages were collected from Mangaldai College, Assam and analysed for phytochemical constituents, antioxidant activity, amino acid content and nutritive value. Except anthraquinone, all the phytochemicals *i.e.* tannin, phenol, flavonoid, terpenoid, cardiac glycoside, alkaloid and reducing sugar were present in all the samples. Highest antioxidant activity was recorded in sample1 (stage 1) followed by sample 2 (stage 2). Considerable amount of nutritive value was found in all the samples and sample 2 showed highest values (287.78 cal/100 g). Different amino acid, *i.e.* cystein, glycin, phenylalanine, serine, valine and glutamic acid were recorded from the samples. The results revealed that both primary and secondary metabolite content of the fern varies with its growing stage.

Key words : *Antioxidant activity, Nutritive value, Amino acid, Phytochemical, Metabolite.*

Introduction

The use of locally accessible therapeutic plants by individuals for treating various afflictions is a typical act of humanity and has been drilled since a long time. The interest in common hotspots for misery, palliative or therapeutic treatments for various sicknesses or recreational use returns to the most punctual purposes of history. Plants are profoundly equipped for synthesising aromatic compounds, dominatingly secondary metabolites of which 12,000 are isolated, a number assessed to be under 10% of the aggregate (Mallikharjuna *et al.*, 2007). Scientific exploration has permitted us to perceive a wide scope of dynamic parts, the most critical of which are oils, alkaloids, flavonoids, phenolic mixes, glycosides, terpenoids, tannins, and so forth (Okwu, 2005). Lately, there has been an overall pattern towards the utilization of the regular phytochemicals present in berry crops, teas, spices, oilseeds, beans, foods grown from the ground (Kitts *et al.*, 2000; Muselik *et al.*, 2007; Jiao *et al.*, 2000). There are around 1250 types of plants and greenery partners known from India, of which 414 types of

greeneries and plant partners from the Western Ghats, India, have been recorded. Of these, 219 greeneries are in danger, 160 are fundamentally jeopardized, 82 are almost imperilled and 113 are uncommon (Manickam and Irudayaraj, 1992; Chandra *et al*., 2008). As estimated by WHO around 70-95% of global population particularly in developing countries use traditional, complementary, alternative, or non-conventional medicines for their health care. *Diplazium esculentum* (Retz.) Sw. is the most commonly consumed fern in hill tribes of North Eastern India along with Bangladesh and Philippines. It is commonly known as *'dhekiashak'* in Assam and mostly in river side and moist areas at dry sites. *Diplazium*is a pantropical fern genus of about 350 to 400 species. *Diplazium esculentum* (Retz.) Sw.is regarded as the most important edible fern worldwide (Useful Tropical Plants, 2020). Young leaves of the plants *Diplazium esculentum* (Retz.) Sw. are cooked as vegetables by the tribal's in Indian mountains (Yumkham and Singh, 2011; Sen and Ghosh, 2011). Amit and Singh (2012) uncovered the Anthelmintic properties of *Diplazium esculentum* (Retz.) Sw. The young fronds rich in iron, phosphorus, potassium and protein are stir-fried as vegetable or used in salads. It is believed by the native tribes of India that the plant counteracts constipation and is used as appetizer. Besides, the tribal communities, ethnic groups and folklore throughout the world are utilizing various parts of the plants like rhizome, stem, frond, pinnae and spores in various ways for the treatment of various ailments since ancient times. (Junejo *et al.,* 2015). Kirtikar *et al.,* (1935) have depicted 27 types of greeneries having different therapeutic employments. Chopra, (1933) have included 44 species and Nadkarni, (1954) recorded 11 types of Pteridophytes having therapeutic significance. Nayar, (1959) uncovered the 29 therapeutic greeneries. May, (1978) distributed an itemized survey of the different employments of greeneries furthermore, recorded 105 therapeutic greeneries. Crude ethanolic concentrates of *Diplazium muricatum* (Mett.) Alderw, *Diplazium travancoricum* Bedd. and *Diplazium brachylobum* (Sledge) Manickam and Irudayaraj are utilized as broad spectrum bioactive agent after extensive investigations (Sivaraman *et al., 2011*). In the methanol extracts of five ferns in *Pteridaceae* family such as *Pteris argyreae* T. Moore, *Pteris confusa* T.G. Walker, *Pteris vittata* L., *Pteris biaurita* L., and *Pteris multiaurita* Ag., by both qualitative and quantitative screening methods confirmed the presence of alkaloids, flavonoids and saponins (Gracelin *et al.,* 2013).

Materials and Methods

Collection of Plant Material: Plants were collected at four different stages from nearby area of Mangaldai College, Assam. These four stages are 1) Stage1- Fiddlehead young twig. 2) Stage 2- Young twig. 3) Stage 3- Premature twig 4) Stage 4- Mature twig. Collected plants were washed with water and shade dried and powdered. The powdered was macerated in methanol for 48 hours and filtered using Whatman No.1 filter paper. The filtrate was evaporated in water bath at a constant temperature of 72 °C until a very concentrated extract was obtained. The crude extract was dissolved in DMSO to make a final concentration, which was kept in the refrigerator till used.

Phytochemical analysis: Phytochemical analysis *i.e.*, for tannin, saponin, flavonoid, phenol, cardiac glycoside, alkaloid, anthraquinone, trapezoid, steroid, reducing sugar of all the four different stages of *Diplazium esculentum* (Retz.) Sw. was determined by following the methods of Tongco *et al.,* (2014).

Antioxidant activity: The DPPH free radicals scavenging activity of the sample was done using the method described by Stanojevice *et al.,* (2009).

Determination of nutrient content: Nutritive value was determined by the following formula Indrayan *et al.,* (2005).

Nutritive value = 4 × percentage of protein + 4 × percentage of carbohydrates + 9 × percentage of fats

Determination of amino acid: Amino acid detection was done by paper chromatography method Thompson and Morris, (1959).

Results and Discussion

The quantitative analyses for phytoconstituents of the samples are summarized in Table 1. Presence of alkaloid, tannin, saponin, trapezoid, flavonoid, phenol, cardiac glycoside, steroid, reducing sugar and absence of anthraquinone were confirmed in all the samples. Generally, the phenolic content largely flavonoid in nature is found to be high in *Diplazium esculentum* of Philippines (Jovale *et al.,* 2014). In this study we confirmed absence of anthraquinone, but Choudhury *et al*., (2017) reported anthraquinone in *Diplazium esculentum* (Retz.) Sw. collected from the wetlands of Dakshin Dinajpur districts of West Ben-

Table 1. Phytochemical screening of extracts of four different stages of *Diplazium esculentum* (Retz.) Sw.

	Sample			
Parameter	Sample 1	Sample 2	Sample 3	Sample 4
Tannin				
A) $FeCl_3$test	+	+	+	+
B) $PbAc_3$ test	+	+	+	+
Saponin	+	+	+	+
Flavonoid	+	+	+	+
Phenol	+	+	+	+
Alkaloid	+	+	+	+
Anthraquinone	-	-	-	-
Cardiac glycoside	+	+	+	+
Trapenoids	+	+	+	+
Steroids	+	+	+	+
Reducing sugar	+	+	+	+

('+') indicates presence; while ('-') stands for absence (Values are means of triplicate determination (n = 3) ± standard deviation).

gal, India. Again, unlike the experimental findings by Choudhury *et al*., (2017), we found cardiac glycoside, tannin, steroid, amino acid present in our study. In case of antioxidant activity, methanolic extract of the samples showed effective scavengers of DPPH radical (Table 2). The percentage inhibition in case of DPPH was 5%, 18.91%, 19.8%, and 21.5% for sample 1, 2, 3, 4 respectively. Highest antioxidant activity (21.5%) was noted in sample 4. Semwal *et al.,* 2013 found the IC_{50} of the methanolic extracts ranged between 0.32 ± 0.12 and 0.81 ± 0.21 mg/ml respectively and Akter *et al.,* 2014 found the IC_{50} of chloroform extract and methanolic extract of *Diplazium esculentum* (Retz.) Sw. was 95669.52 mg/ml and 5907.53 mg/ml respectively. Choudhary *et al.,* (2017) found that IC_{50} value of methanolic extract of *Diplazium esculentum* (Retz.) Sw. was 3.8 mg/ml. The highest nutritive value (287.78 cal/100g) was obtained in sample 2 followed by sample 4, 3 and 1. The different type of amino acid was obtained from different stages of *Diplazium esculentum* (Retz.) Sw. such as Cystein, Serine, Valine, Glycine, Alanine, Phenylalanine. Choudhary *et al.,* (2017) in their study found 26.50 ± 1.41 mg/mg dwt of protein and 35.00 ± 0.32 mg/g dwt. total sugar. Chettri *et al.,* (2018) found 89.34% moisture, 0.25% crude fat, 1.33% ash and 3.84% protein in *Diplazium esculentum* (Retz.) Sw. collected from different localities in the Sikkim Himalayas.

Table 2. Antioxidant activity (%inhibition in mg/ml) of four different stages of *Diplazium esculentum* (Retz.) Sw.

Sample	Antioxidant activity (% inhibition in mg/ml)
Sample 1	5%
Sample 2	18.91%
Sample 3	19.8%
Sample 4	21.5%

The present study shows presence of phytochemicals, antioxidant activity and good amount of nutritive value and amino acid in the samples of *Diplazium esculentum* (Retz.) Sw. The variation in antioxidant activity, amino acid and

Table 3. Nutritive value of four different stages of *Diplazium esculentum* (Retz.) Sw. (Values are means of triplicate determination (n = 3) ± standard deviation)

Sample	Ash (%)	Moisture (%)	Crude fat (%)	Protein Content (%)	Carbohydrate (%)	Nutritive Value (cal/100 g)
Sample1	12.74	31	3.2	1.94	51.22	241.44
Sample2	13.08	20	4.02	10.86	52.04	287.78
Sample3	11.90	26.5	2.91	12.84	45.85	260.95
Sample4	11.65	21	3.43	12.31	51.7	286.91

Table 4. List of amino acids present in different stages of *Diplazium esculentum* (Retz.) Sw.

Sample	Amino acid
Sample1	Cystein, Serine, Valine
Sample2	Glycine, Valine,Alanine
Sample3	Phenylalanine, Glycine
Sample4	Phenylalanine, Glutamic Acid

nutrient content in four different stages of *Diplazium esculentum* (Retz.) Sw. was recorded in the present study. Again it is also found that variation is also found in the antioxidant activity, nutrient content etc in the present study and earlier study conducted by Choudhury *et al.,* (2017) and Chettri *et al.,* (2018), Aktar *et al.,* (2014). Thus, the production of secondary metabolite of a plant may vary both qualitatively and quantitatively with respect to its growing conditions. In fact, this issue needs to be properly addressed in case of medicinal plants and is of paramount importance in quality control of crude drugs. The study also provides scientific basis of the use of the plants at different stages belonging to same species collected from Darrang district, Assam.

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