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Determination of Phytochemicals, Minerals, and Evaluation of Antioxidant, Antidiabetic Activities of Young Fronds of *Diplazium esculentum*: A Vegetable of Tripura, India

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Abstract

Diplazium esculentum belongs to the family Athyriaceae, an edible fern rich in micronutrients, beta-carotene, folic acid, and minerals like calcium, iron, and phosphorus. The present study aims to determine secondary metabolites and minerals content and evaluate the antioxidant and antidiabetic activities of young fronds of *Diplazium esculentum*. The plant extract was prepared by the Soxhlet apparatus method. Total alkaloids and total tannin content were determined by the titrimetric method. Folin and Ciocalteu's colorimetric method used gallic acid as a standard drug to determine total phenols. The antioxidant activity of plant extract was evaluated by DPPH free radical scavenging and hydrogen peroxide scavenging assay. α-amylase inhibition assay was performed for evaluation of the antidiabetic activity of plant extract. Fronds of *Diplazium esculentum* contain a significant number of total alkaloids (145.54±1.49), phenols (174.2±0.95), and tannins (263.96±1.32) mg/g of dry extract. Fronds of *Diplazium esculentum* also contain a significant amount of minerals. *Diplazium esculentum* shows significant antioxidant activity compared to the standard drug ascorbic acid and antidiabetic activity compared to the standard drug metformin. Based on these findings, young fronds of *Diplazium esculentum* can be recommended as a good source of phytochemicals and minerals. It also shows significant antioxidant and antidiabetic activities.

Keywords: *Diplazium esculentum*; Phytochemicals; Minerals; Antioxidant activity; Antidiabetic activity.

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1. Introduction

Since ancient times, medicinal plants have been widely used to treat diseases and ailments [1]. The North-Eastern region of India is home to several medicinal plants. However, many of these have not yet been scientifically explored or validated for their proclaimed efficacies [2]. *Diplazium esculentum* belongs to the family Athyriaceae [3], an edible fern

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rich in micronutrients, beta-carotene, folic acid, and minerals like calcium, iron, and phosphorus [4]. *Diplazium esculentum* has been found to be effective against headache, pain, fever, wounds, dysentery, glandular swellings, diarrhea, and various skin-related infections [5]. Young fronds of *Diplazium esculentum* are used as vegetable salad [6]. In Tripura, a state in northeast India, it is locally known as "Dheki Shak," which grows with an average height of 0.5 to 2.5 m [7,8]. During its early stage, the stipe is around 20-30 cm long and looks pale brown. As the plant ages, the stipe becomes smooth [9]. Protrusions are also sometimes observed near its base. The leaves are found to be oblong-lanceolate with an 80-130 cm approximate length. Bulbils are also observed, which are scaly and are seen near the base of the plant [10]. It is being widely distributed in South Asia countries like India, China, Cambodia, Laos, Vietnam, and Malaysia. It grows in large clusters with favorable environments like open muddy, damp areas, stream banks, and canals [11]. People of various tribes of Tripura exploit it for its medicinal value [12,13]. The pharma scientist is also motivated to formulations of plant-derived products for better efficacy and the least adverse effect. Research reveals that natural antioxidant agents are being accepted for managing diabetes [14]. Thus, the main objectives of our present study were the determination of secondary metabolites and minerals and the evaluation of the antioxidant and antidiabetic activity of young fronds of *Diplazium esculentum*: A vegetable of Tripura, India.

2. Study Objects and Methods

2.1. *Plant material collection*

Young fronds of *Diplazium esculentum* were collected from a local cultivator at the village of Netajinagar, Teliamura, Khowai district of Tripura, Northeast India, in the month of July 2019. The sample was identified by B. K. Datta, a taxonomist at the Department of Botany, Tripura Central University, Suryamaninagar-799022, India.

2.2. *Processing of the plant's material*

Young fronds of *Diplazium esculentum* were washed thoroughly with tap water cut into small pieces, and air-dried (applied warm temperature, low humidity with an air current) completely. Then the dried pieces were further processed for grinding to obtain a fine powder and passed through a sieve of mesh size 40. This powder material was packed in an airtight container and stored at room temperature for further experiment [15].

2.3. *Preparation of plant extract*

Dried fronds powder of *Diplazium esculentum* (30 g) were packed into a Soxhlet apparatus and extracted with 300 mL 50 % ethanol at 55–60 °C for 72 h. The extract was filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure at 40 °C in the rotary vacuum evaporator (IKA HB 10). The extract was

dried through a water bath at 40 °C temperature, weighed, and stored at 4 °C in storage vials for experimental use. The percentage of extract yield of 12 % was determined by the following equation [16].

$$\%\, of yield = \frac{Weight\ of\ the\ extract}{Weight\ of\ the\ plant\ material} X\ 100$$

2.4. *Qualitative analysis of plant secondary metabolites*

Fronds extract of *Diplazium esculentum* (stored at 4 °C) had been dissolved initially with 50 % ethanolic solution at 1 mg/mL concentration. For quantitative analysis from this stock, 2 mL of the solution has been withdrawn.

2.4.1. *Test for alkaloids*

Wagner's Test: 2 mL of test solution, when added with a few drops of Wagner's reagent (iodine in potassium iodide) from the periphery of the test tube, yellow or brown residue infers that the alkaloid is present [17].

2.4.2. *Test for phenols*

Ferric chloride test: 2 mL of test solution, when added with a bit of amount of 5 % (w/v) aqueous ferric chloride solution, a deep blue or black color, shows the presence of phenolic compounds [18].

2.4.3. *Test for tannins*

Braymer's test: 2 mL of test solution mixed with a small amount of 10% alcohol ferric chloride solution produces a blackish-blue color or green-blackish color, identifying the presence of tannin [17].

2.5. *Quantitative analysis of plant secondary metabolites*

2.5.1. *Determination of total alkaloids*

Alkaloid was determined by the acid-base titrimetric method. Fifty milligrams of plant extract were taken and dissolved in 20 mL of n-butanol. Then 10 mL of extract solution was piped into a separating funnel, and 10 mL of 0.1 (N) HCl was added. After that, the solution was shaken vigorously for a few minutes. Then the solution was kept for 20 min undisturbed until the two layers had separated. The denser layer was collected from the separating funnel in 100 mL conical flax and added few drops of methyl red indicator. This solution was titrated against 0.1 (N) NaOH until the color changed from pale pink to pale yellow, and the endpoint was noted. For blanking, 10 mL of 0.1 (N) HCl and a few

drops of methyl red were added to 100 mL conical flax and titrated against 0.1 (N) NaOH until the color changed from pale pink to the pale-yellow endpoint, which was noted [19]. The total amount of alkaloids was calculated by considering the following equivalent:

1 mL 0.1(N) HCl ≡ 0.01629 g of alkaloids

2.5.2. *Determination of phenolic content*

Folin and Ciocalteu's colorimetric method was used to determine the phenolic content of the plant extract. A standard stock solution (1 mg/mL) of gallic acid was prepared with the help of methanol. Then the stock solution was diluted ten times. From this stock solution, five different concentrations (0.01, 0.02, 0.03, 0.04, 0.05 mg/mL) were pipette out in individual test tubes. Then make up to 3 mL with distilled water and mix thoroughly with 0.5 mL of Folin and Ciocalteu reagent. After that, the reaction mixture was incubated for 3 min at room temperature. Then added 2 mL of 35 % (w/v) sodium carbonate and incubated for 45 min in the dark at room temperature. Finally, the absorbances of the reaction mixture were taken at 650 nm in a calibrated UV–visible spectrophotometry (Shimadzu UV-1800). For the sample (triplicate), 200 µL was taken, and the color was generated as usual. The calibration curve helped to determine the total phenolic content in the extract.

2.5.3. *Determination of tannin content*

The total tannin content of the plant extract was determined by using the titrimetric method. Seventy-five milligram of plant extract was taken and dissolved in 150 mL of 50 % ethanol (stock solution). From this solution, 12.5 mL was taken out into a conical flask, 12.5 mL of indigo solution was added as an indicator, and 375 mL of distilled water. The whole solution was titrated against 0.1 (N) $KMnO_4$ solution until the color changed from blue to green. Further added a few drops of titrant changed into golden yellow color, and the borate reading was noted down. For blanking, 12.5 mL of 50 % ethanolic solution (without sample) was taken in conical flax, 12.5 mL of the indigo solution was added to it and finally added 375 mL with distilled water. It was titrated as the same and noted the endpoint. The following equation was used for the determination of tannin content [21].

$$The\ tannin\ content(T\ \%) = \frac{(V - V0)X\ 0.004157\ X\ 150X\ 100}{gx\ 12.5}$$

Where, V= 0.1 N aqueous solution of $KMnO_4$ for the titration of the sample, mL.
V_0= 0.1 N aqueous solution of $KMnO_4$ for the titration of the blank sample, mL.
0.004157= Tannin equivalent in 1 mL of 0.1 N aqueous solution of $KMnO_4$.
g= Mass of the sample taken for analysis.
150 = Volume of the stock solution, mL.

2.6. *Determination of minerals*

2 g of powder sample was weighed and transferred in a Teflon digestion vessel with 65 % nitric acid (28 mL) and 30 % hydrogen peroxide (1 mL). After that, the sample vessel was placed in a microwave for 10 min at 200 °C. Filtered the digested sample through Whatman filter paper no. 40, and the volume was made up to 100 mL with deionized water. This solution was used to analyze minerals (iron, sodium, potassium, calcium, and magnesium) using an atomic absorption spectrophotometer (Perkin Elmer AAS 700 associated with MHS-15 hybrid generator) [22].

2.7. *Antioxidant activity*

2.7.1. *DPPH free radical scavenging assay*

Antioxidant activity of *Diplazium esculentum* fronds was carried out by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay with a slight modification of the Sowndhararajan and Kang (2013) method. A standard stock solution of ascorbic acid (1 mg/mL) was prepared with the help of methanol. Various concentrations (10, 20, 40, 80, and 100 µg/mL) were taken in different test tubes from this stock solution, and added an equal volume of methanolic DPPH solution (0.1 mM), then incubated the reaction mixture for 30 min in a dark room at room temperature. After half an hour, the absorbance was measured at 517 nm against the blank methanol. 0.1 mM DPPH solution was used for control. The following equation has been applied to determine the percentage inhibition of ascorbic acid [23].

$$DPPH\ scavenging\ effect = \frac{Control\ OD - Sample\ OD}{Control\ OD}\ x\ 100$$

The same procedure was followed to determine the percentage of scavenging of the sample (fronds of *Diplazium esculentum*).

2.7.2. *Hydrogen peroxide scavenging assay*

Hydrogen peroxide scavenging activity was estimated according to the method reported by Ruch *et al*. (1989), with minor modifications. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). A standard solution of 1 mg/mL ascorbic acid was prepared in phosphate buffer (pH 7.4). From this stock solution, 10, 20, 40, 80, and 100 µg/mL were taken into separate test tubes, and 0.6 mL of hydrogen peroxide (40 mM) solution was added and incubated for 15 min at room temperature in dark conditions. Absorbance was recorded at 230 nm. Percentage inhibition was calculated by the following equation [24].

$$Hydrogen\ peroxide\ \ scavengingeffect = \frac{Control\ OD - Sample\ OD}{Control\ OD} x\ 100$$

The same procedure was followed to determine the percentage of scavenging of the sample (fronds of *Diplazium esculentum*).

2.8. *α-Amylase inhibition assay*

Antidiabetic activity of plant extract was carried out method reported by Ali *et al*. (2006), with minor modifications. A stock solution of metformin (1 mg/mL) was prepared in methanol. From this standard (stock) solution, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL were taken in five test tubes, and 0.5 mL α-amylase enzyme solution was mixed. Then the mixture of different concentrations has incubated for 30 min at 25 °C±2 °C. After that, 0.5 mL of the solution was pipette out to every test tube and added 0.5 mL potato starch (0.5 % w/v), and incubated for 3 min at room temperature. After incubation, 0.5 mL of DNSA reagent was added. Again, incubate the all-reaction mixture for 15 min at 85 °C. After 15 min, the test tubes were cooled properly and made up to the volume of 5 mL with distilled water. The absorbance was noted down at 540 nm [23]. DNSA reagent and distilled water mixed in a proper ratio were used as a blank. For control, drugs have not been added to the reaction mixture [25].

$$Inhibition\ of \alpha - amylase\ = \frac{Control\ OD - Sample\ OD}{ControlOD} x\ 100$$

3. Statistical Analysis

Statistical analysis was conducted on the statistical software package SPSS (SPSS 15.0 software). The mean and standard deviation of the mean (SD) values of secondary metabolites and minerals were determined. Evaluation of antioxidant activity by DPPH, H_2O_2, and antidiabetic activity by α-amylase inhibition assay standard error mean (SEM) was calculated for each concentration.

4. Results and Discussion

4.1. *Qualitative and quantitative analysis of secondary metabolites*

The qualitative and quantitative analysis of *Diplazium esculentum* frond is represented in Table 1.

Table 1. Qualitative and quantitative response of secondary metabolites of *Diplazium esculentum.*

Name of the phytochemicals	Qualitative response	Quantitative amount (mg/g of dry extract)
Alkaloids	++	145.54±1.49
Phenols	++	174.2±0.95
Tannins	+++	263.96±1.32

"++" = Moderate concentration; "+++" =High concentration.
* Each value represents the mean ± SDs calculated as dry weight (DW) basis in triplicate.

It has been disclosed from the literature that the majority of the bioactive compounds dissolve in 50 % ethanol [26]. From the current experiment, it was found that the frond of *Diplazium esculentum* gave a good quantity of extract yield (12 %). Medicinal plants have

a vital role in different curative types of ailments in human and animal bodies due to the presence of secondary metabolites [27]. Alkaloids are secondary metabolites of plants containing nitrogen, usually in a ring. In a small quantity of alkaloids used as medicine but in large amounts, it has been toxic in nature [28]. Alkaloids have shown anticancer, CNS stimulant, anesthetics, cardioprotective, and anti-inflammatory activities. Phenols and tannins are two important phytochemicals that give antioxidant, anti-inflammatory, and cardioprotective activities [29]. Tannins also use as an astringent [30]. In the present experiment, it was observed that fronds of *D. esculentum* contain a good amount of alkaloids, tannins, and phenols. So, the edible parts of *D. esculentum* may give pharmacological activities in human and animal bodies.

4.2. *Determination of minerals*

The total mineral content (iron, sodium, potassium, calcium, and magnesium) of fronds of *D. esculentum* is represented in Table 2.

Table 2. Minerals profile of fronds of *Diplazium esculentum.*

Minerals	Fronds of *Diplaziumesculentum* mg/100 g of dry powder (Mean±SDs)
Iron	13.1±1.73
Sodium	11.66±2.15
Potassium	923.03±7.63
Calcium	192.26±3.47
Magnesium	0.44±0.09

* Each value represents the mean ± SDs calculated as dry weight (DW) basis in triplicate.

Calcium, sodium, potassium, magnesium, and iron are five essential minerals in the human body. Calcium helps to build strong bones and teeth [31]. Sodium and potassium play a vital role in maintaining fluid balance, muscle contraction, and nerve impulse conduction [32]. Magnesium controls protein synthesis, muscle and nerve function, and blood glucose regulator [33,34]**.** Iron helps to maintain the hemoglobin level in blood. A deficiency of iron decreases the oxygen level in blood and causes cell death [35,36]. Fronds of *D. esculentum* contain a good quantity of these five essential minerals. So, it may be able to regulate the diseases related to the deficiency of these five essential minerals.

4.3. *Evaluation of antioxidant activity*

The Frond of *D. esculentum* showed significant scavenging activity compared to standard ascorbic acid against the DPPH and H_2O_2 scavenging assay (Fig. 1). IC_{50} values of the antioxidant activity are represented in Table 3.

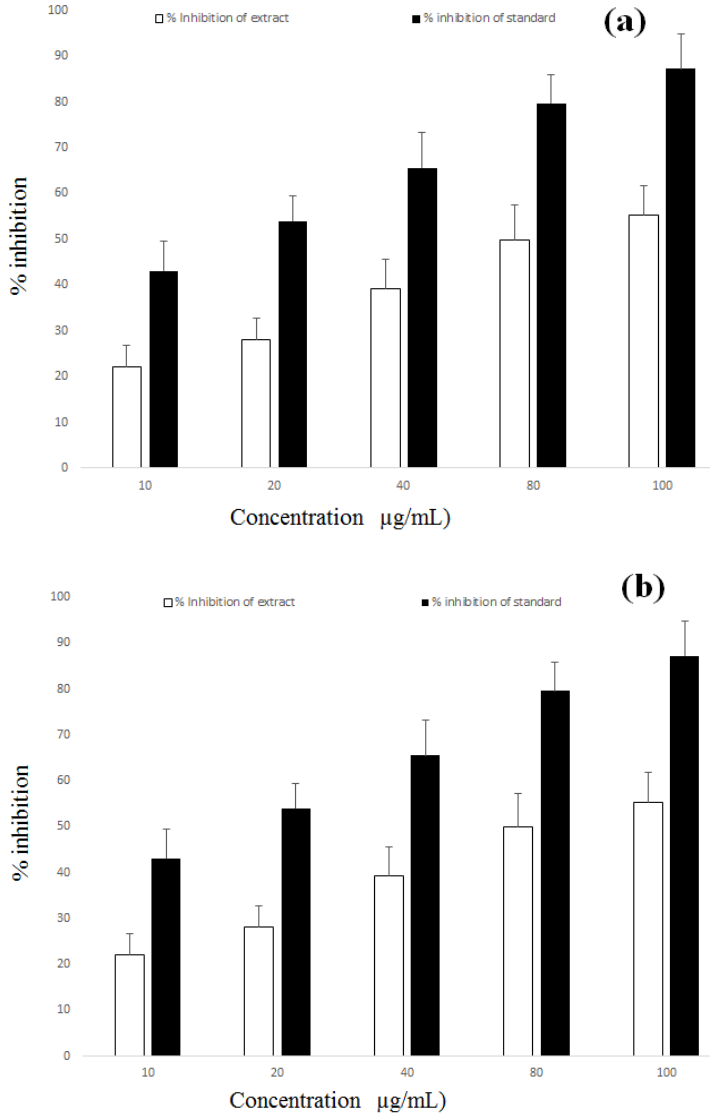

Fig. 1. DPPH free radical scavenging assay (**a**), Hydrogen peroxide scavenging assay (**b**) of *Diplazium esculentum* fronds. Ascorbic acid was used as a positive control.

Table 3. The half-maximal inhibitory concentration of DPPH and H_20_2 scavenging assay.

Name of the sample	IC_{50} value (µg/mL)	
	DPPH scavenging assay	H_2O_2 scavenging assay
Fronds extract	66.943	68.02
Ascorbic acid (standard)	44.00	48.09

Free radicals play a crucial role in the pathogenesis of various diseases, especially degenerative diseases and extensive lysis. They constantly degenerate in the living system and are responsible for damaging cellular biomolecules, such as proteins, enzymes, nucleic acids, lipids, and carbohydrates, adversely affecting immune function [37]. Antioxidants inhibit the production of free radicals and play a key role in activating them [38]. All human cells protect themselves against oxidative damage by various antioxidant mechanisms, but sometimes these are insufficient to prevent the damage caused by free radicals. Therefore, natural food supplements are used to protect against oxidative damage [39]. In the present study, the total antioxidant capacity of the young frond extract of *D. esculentum* was measured by a DPPH and H_2O_2 free radical scavenging assay and found significant antioxidant activity.

4.4. *Evaluation of α-amylase inhibition activity*

The fronds of *Diplazium esculentum* showed significant inhibition of *the α-amylase* enzyme compared to the standard drug metformin (Fig. 2). The IC_{50} values of α-amylase inhibition of frond of *Diplazium esculentum* and metformin are 742.94 µg/mL and 523.01 µg/mL, respectively.

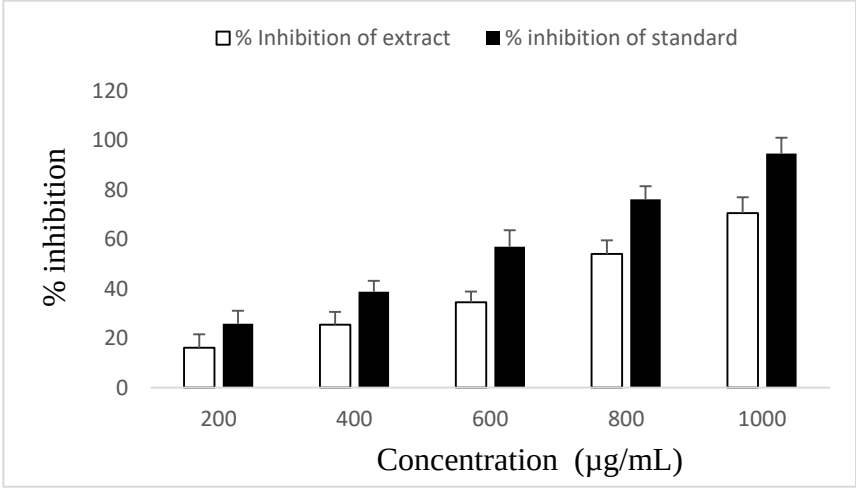

Fig. 2. α-Amylase inhibition activity of *Diplazium esculentum* fronds.

The hypoglycemic effect of any natural products is observed due to dietary polyphenols, which act by getting bonded to glucose transporters by competitive inhibition with digestive enzymes [40]. α-Amylase acts by digesting the carbohydrates, thereby increasing the post-prandial glucose level in diabetic patients. Thus, the inhibition of alpha-amylase activity reduces the post-prandial glucose level [41]. In this research work, antidiabetic activity was performed by inhibiting the alpha-amylase, and the result showed significant inhibition activity.

5. Conclusion

Based on these findings, it was observed that the young frond of *Diplazium esculentum* contained a good amount of phytochemicals and minerals. It also shows significant antioxidant and antidiabetic activities.

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