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ANALYSIS OF NUTRITIONAL COMPOSITION AND QUANTITATIVE ANALYSIS OF AQUATIC FERN AZOLLA PINNATA

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ABSTRACT

Now- a days our food and lifestyle habits have resulted in the development of number of diseases and disorders, so paramount percentage of the inhabitants depends on folk medicines. So, an attempt has been made to analyses the nutritional composition and quantitative analysis of Azolla pinnata. Azolla is a pteridophyte belonging to the family Salviniaceae having idyllic importance in developing as well as in developed countries. It acts as a biofertilizer, and increases the productivity of rice. As Azolla is a good source of protein, and it contains all essential amino acids, fatty acid, vitamins and minerals such as iron, calcium, magnesium, potassium, phosphorus etc. Whole part of Azolla were tested for proximate constituents. The moisture content was found to be 84.3±0.20, ash value was found to be 16.59±0.20, extractive value were found to be different in different solvents. The quantitative analysis of Azolla pinnata disclosed the presence of significant quantity of protein that was 26.72±2.15mg/g of anthrone equivalents, flavonoids that was 50.54±1.83mg/g of quercetin equivalents, tannins that was 80.28±2.17mg/g of tannic acid equivalents, carbohydrate that was 42.68±4.316mg/g of glucose equivalents and phenol content that was 87.69±2.85 mg/g of GAE.

Keywords: Azolla, proximate composition, carbohydrate, proteins, flavonoids, tannins , phenol.

1. INTRODUCTION

In developing countries there is always an ever ending exploration for the accessibility of nutritionally rich and inexpensive food resources. Aquatic plants are attaining much interest in food and biomedical research such as human food, animal feed and bio-fertilizers. Due to changes in lifestyles, food habits and nature of work in recent days the incident of serious diseases like diabetes, obesity, heart attacks are more recurrent in younger generation. This circumstances evidently calls for the search for medicinally agile along with nutritionally rich non traditional food sources. Azolla is one such genus belonging to family Salviniaceae which grows in association with blue green algae Anabaena azollae, a nitrogen fixing organism**.**

Azolla is an aquatic free floating fern belonging to the family Salviniaceae. It has multiple significance as it produces utmost biofuel in a momentary period of time . It acts as a biofertilizer. The fern Azolla come out to be successively eminent as a biofuel on version of its soaring development pace, production of biogas with elated amounts of biofuel-generating capacity. Apart from this Azolla has been seem to be fit for hyperactive accruable a phenomenal assortment of massive metal toxins and in addition to this it also refining the phosphorous and ammonia in wastewater . It is used as a food for humans, feed for animals, bio-fertilizer, hydrogen fuel, biogas production, water purifier, weed and bug controller(.

From the point of view it is considered as the most promising because of its nutritive value, ease of cultivation and human consumption. Traditionally now it is used as a livestock feed, nutritional supplement and bio-fertilizer . It is a revenue making crop. It is effortlessly cultivated but requires sufficient stagnant water, relative humidity of 85-90%, salinity between 90-150mg/l, pH of 4.5-6.5. It grows very fast and within no time it double its weight. Azolla pinnata was used as a feed grain for laying hens, broiler chicken , goats , juvenile black tiger shrimp and buffalo calves .while, Azolla filiculoides was also used as a diets for sows and it was also used as a partial substitution of protein source for growing caloric pigs. Azolla is rich in protein, almost all essential amino acids, minerals, vitamins. It also contains biopolymers and probiotics. It strengthen the quality of water by banishing the phosphorous and nitrates . Analyzing the significance of the organism as a dietary supplements there is a requirement to explore the carbohydrate, protein, flavonoids, phenol and tannins profile of this aquatic fern.

Very less studies were done to investigate its nutritional profiling and quantitative analysis. Hence this present study was carried out to evaluate its proximate nutritional chemical composition and quantitative analysis.

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2. MATERIALS AND METHODS

Plant Collection and Preparation Of Extract

Plant collection: Whole plant parts of Azolla pinnata were collected from Divyan, Morabadi, Ranchi, Jharkhand.

Fig.1. Collection of Azolla pinnata

Fig. 2. Drying of Azolla leaves

Preparation of extract: The plant material was washed thoroughly under tap water to remove all the soil particles, mud, dirt from it. After that rinsed it with distilled water. The plant material was allowed to shade dried at room temperature for 3-4weeks until it was completely dried. The dried material was then grind to made a coarse powder of it using mortar and pestle. The grind sample was subjected to extraction by cold maceration using solvents methanol to obtain extracts. 5gm of powder were added in 50ml of solvent and stirred it occasionally in rotary shaker and kept it for 48hrs, after 2 days the mixture were filtered by using whatman filter paper. After filtration each solvent was allowed to evaporate at room temperature for 18-24hrs to obtain a solid mass. The solid mass which was obtained were also stored in refrigerator at 4˙c for further use.

3. DETERMINATION OF PROXIMATE COMPOSITION IN AZOLLA PINNATA

Moisture content: Moisture content in Azolla was determined by heating the plant powder in hot air oven at 120℃. The difference between the initial weight of plant powder and the final weight after drying the plant powder is the moisture content.

Ash content: The ash content was determined by heating the plant powder in an earthen pot for an hour continuously on heater. All the foreign matters and moisture were removed, and cellular parts gets degraded and only cellular residues were present. Finally, it was weighed to get ash value.

Extractive value: The extractive value was determined by soaking the plant powder in five different solvent and mixed it properly and covered it with aluminum foil and left it for 48hrs.

The extract was filtered in five different weighed beakers. The liquid extract was left to evaporate, after evaporation weight of the beaker was measured.

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QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Quantification Of Total Flavonoid Content

The total flavonoid content present in the extract was determined by using Aluminium chloride method. In 0.5ml of extract, add 3.4ml of 30% ethanol followed it with by adding sodium nitrate and aluminum chloride and mixed it very well. After 5 minutes, add 1ml of sodium hydroxide and the solution was mixed properly. The absorbance was measured at 510nm. A standard calibration curve was constructed by using quercetin of different concentrations. The total flavonoid content was expressed as mg of quercetin equivalent/g.

Quantification Of Total Tannins Content

In a series of test tubes 0.5-1ml of standard tannic acid solution was pipette out. About 0.5ml of extract solution was taken in another test tube. By adding distilled water make the volumes of all the test tubes up to 3ml. 3ml of distilled water was taken as a blank also. In all the test tubes added 5ml of 20%Na_2CO_3 followed by the addition of 2.5ml of Folin-Dennis reagent. Incubate it for 30minutes at room temperature and the bluish-green color was developed at 700nm. From the standard curve the amount of tannin present in the extract was calculated and the result was expressed as mg tannic acid equivalent/g.

Quantification of Total Protein Content

The total protein content was determined by using Biuret method. In a series of test tubes pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1ml solution. In an another test tube pipette out 1ml of given sample. Make it up to the volume of 1ml of each test tubes. Tube with 1ml of distilled water serves as a blank. Now add 3ml of Biuret reagent in all the test tubes including the blank and unknown named test tubes. Mix the contents of the test tubes and warm it at 37˚c for 10 minutes. Now cooled the contents at room temperature and record the absorbance at 549 nm against blank. Plot the standard curve. From this standard curve calculate the concentration of protein in the sample.

Quantification of Total Carbohydrate content

The total carbohydrate content was determined by Anthrone reagent method. In a series of test tubes pipetted out different volumes of glucose solution from the stock solution to make upto the volume of 1ml by adding distilled water into it. Kept tube 1 as a blank tube and rest tubes were used for the construction of standard curve. In each test tubes add 5 ml of anthrone reagent and mixed it. Now cool the test tubes. Cover the test tubes with cotton plug and incubated it at 90·c for 10-15 minutes. Cool the test tubes at room temperature and measure the optical density at 620nm against the blank.

Quantification of Total Phenol content

The total phenol content was determined by using Folin-ciocalteu method. Standard taken here was gallic acid and the total phenol content was calculated as gallic acid equivalents. The absorbance was measured at 760 nm. A standard calibration curve was constructed by using gallic acid of different concentrations. The total phenol content was expressed as mg Gallic acid equivalents/g.

4. RESULT AND DISCUSSION

Proximate Composition of Azolla pinnata

In the present investigation the proximate analysis of Azolla showed that it contains 84.3±0.20 dry matter, 16.59±0.20 ash value, 5.2±0.20 methanol soluble extract, 3.5±0.20

ethanol soluble extract, 0.5±0.20 chloroform soluble extract, 2.5±0.20 benzene soluble extract, 9.7±0.20 distilled water soluble extract.

Table 1. Proximate composition of Azolla pinnata

S.no.	Parameters	Result
1.	**Moisture content/Dry matter**	84.3±0.20
2.	**Ash content**	16.59±0.20
3.	**Extractive values**	
a)	Methanol soluble extract	5.2±0.20
b)	Ethanol soluble extract	3.5±0.20
c)	Chloroform soluble extract	0.5±0.20
d)	Benzene soluble extract	2.5±0.20
e)	Distilled water soluble extract	9.7±0.20

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Fig.3. Moisture content

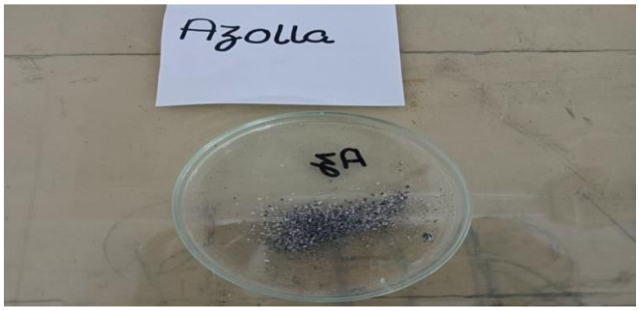

Fig. 4. Ash value

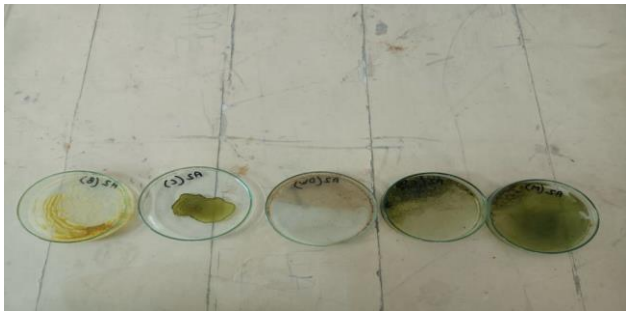

Fig. 5. Extractive value in different solvent

QUANTITATIVE ANALYSIS OF SECONDARY METABOLITES

The study of secondary metabolites in plants is crucial for the extraction, isolation, identification and purification of several drug metabolites.

The result of quantitative analysis indicated the presence of flavonoids, tannins, proteins, phenol and carbohydrate in methanol extract of Azolla. The result of quantitative analysis demonstrated the presence of significant quantities of flavonoids(50.54±1.83mg/g), tannins (80.28±2.17mg/g), protein (26.72±2.15mg/g), carbohydrate (42.68±4.31mg/g) and phenol (87.69±2.85 mg/g).

Table 2. Total flavonoids, tannins, protein, carbohydrate and phenol content in methanolic extract of Azolla pinnata

Extract	Total flavonoid content mg/g of QAE	Total tannins content mg/g of TAE	Total protein content in mg/g of BSAE	Total carbohydrate content in mg/g of GLU	Total phenol content in mg/g of GAE
Azolla pinnata	50.54±1.83	80.28±2.17	26.72±2.15	42.68±4.31	87.69±2.85

5. CONCLUSIONS

Based on the results of this present study, it can be concluded that Azolla could be used as an unconventional food source. It can also be used as a substitute for artificial nitrogen fertilizer. It is advantageous for both farming and animal husbandry. Besides using it as a nutrients in crops it is also used as a fodder as it contains 20-30% of protein, which is much more than any other fodder. It could also be used as a natural protein source in livestock feeds. If you feed Azolla to cows, goats, buffaloes and cattle it will not only increase the milk production by 15-20% but it will also speed up their physical growth. It also saves 40-50% of fodder cost, by mixing it into the feed in the ratio 1:1, so it could be used as a potent feed source to lives.

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