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Phytochemical and Biochemical Profiles of *Azolla microphylla* Cultured with Organic Manure

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Phytochemical and biochemical composition of Azolla microphylla was evaluated. Petroleum ether, water, acetone, methanol, ethanol, chloroform, benzene, methylene chloride extracts were obtained. Phytochemical analysis of A.microphylla revealed the presence of alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, anthraquinones and phenols. Steroids and Tannins were present in most extracts while aromatic acids were absent in all the extracts. Dried and powdered leaves were estimated for protein, lipid, carbohydrate, ash and energy. Protein (0.252mg/g), Carbohydrate (0.178mg/g), Lipid (0.021mg/g) were present. Total ash 48%, acid-insoluble ash 50%, and water soluble ash 80% was also present.

Key words: Azolla, proximate composition, extracts, total ash, energy.

INTRODUCTION

Azolla is an aquatic pteridophyte widely distributed in the water bodies. It has been traditionally used as a biofertilizer for rice paddy fields owing to its potential to fix atmospheric nitrogen. *Azolla* is a genus of six species of aquatic ferns, the only genus in the family Azollaceae. It grows naturally in stagnant water in drains, canals, ponds, rivers and water bodies including marshy lands with temperature range of 15-35°C. *Azolla* has many uses such as animal feed, human food, medicine, production of biogas, hydrogen fuel, water purifier, weed control, reduction of ammonia volatilization and is aptly referred it as green gold mine. The plant system has the inherent capacity to synthesize several biologically active constituents which in turn protect them against the attack of insects and other plant pathogens such as bacteria and fungi. The excessive use of pesticides and chemicals in agriculture and consequent adverse impact on the health has prompted the scientists to look for newer molecules of plant origin. The green chemistry concept is gaining momentum in a big way and several research groups are active in this field of science (Abraham and Vidhu Aeri, 2012). Experiments conducted at Banaras Hindu University, Varanasi showed beneficial effects of *Azolla* in the cultivation of rice (Bhuvaneshwari and Ajay Kumar, 2013). Application of *Azolla* has been found to significantly improve the physical and chemical properties of the soil especially nitrogen, organic matter and other cations such as Magnesium, Calcium and Sodium released into the soil (Bhuvaneshwari, 2012).

MATERIALS AND METHODS

Preparation of the extract

Fresh material was collected from the tanks and were brought to the laboratory and cleaned of all the debris. They were then washed several times in tap water. Subsequently, the plants were washed using double distilled water and were air dried in shade for one week.

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Extracts were then prepared from the dried and powdered materials by boiling them in the respective solvents successively for 16h at a temperature that not exceeded the boiling point of the solvent. The filtrates were then concentrated in vacuum at 45°C. Phytochemical screening was conducted using the standard methods.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the extracts obtained after successively extraction with Ethanol, Methanol, Acetone, Water, Benzene, Petroleum ether, Chloroform and Methylene chloride solvents (Harbone, 1998).

Biochemical parameters

Estimation of protein

The protein content of *Azolla* powder sample was analysed by Lowry method (Lowry *et al*., 1951).

Estimation of lipid

Total lipid content in *Azolla* powder sample was analysed by Bragdon method (Bragdon, 1951).

Estimation of carbohydrate

Total carbohydrate contents of the sample was estimated by the Anthrone method. Carbohydrates are dehydrated by sulphuric acid to form furfural which condenses with anthrone to form blue coloured complex which is measured spectophotometically.

Estimation of energy

Wet combustion method

This is an indirect method of estimating the energy value of the material. The procedure makes use of the principle that potassium iodate when heated in the presence of concentrated H_2SO_4 organic materials decomposes and releases nascent oxygen which insitu oxidizes the organic matter present in the sample. The decomposition of Pottassium iodate is proportional to the energy content of the organic materials.

Physico chemical parameter

The dried plant material was subjected to determination of physicochemical parameters. The ash values such as total ash, acid insoluble ash, water soluble ash was determined according to standard procedures.

RESULTS

Phytochemical analysis of *A.microphylla* revealed the presence of alkaloids, terpenoids, steroids, coumarins, tannins, saponins, flavonoids, anthraquinones and phenols. Steroids and Tannins were present in most extracts while aromatic acids were absent in all the extracts. Ethanol proved to be the best solvent as it contained most of the phytochemical tested while acetone, benzene, petroleum ether and methylene chloride had only four of the phytochemicals tested (Table 1).

Table 1. The analysis of phytochemicals in the different organic extracts of *Azolla microphylla*

S.No.	Test	Ethanol	Methanol	Acetone	Water	Benzene	Chloroform	Petroleum ether	Methylene chloride
1	Alkaloids	+	-	-	-	+	-	-	-
2	Terpenoids	-	-	-	+	-	+	-	-
3	Steroids	+	+	-	+	+	+	+	+
4	Coumarins	+	+	-	-	-	-	-	-
5	Tannins	+	+	+	+	+	-	+	+
6	Saponins	+	+	-	+	-	-	-	-
7	Flavonoids	-	+	-	-	-	-	-	-
8	Quinones	-	-	-	-	-	-	-	-
9	Anthraquinones	+	+	-	-	-	-	-	-
10	Phenols	+	+	+	-	-	+	-	-
11	Aromatic acids	-	-	-	-	-	-	-	-
12	Proteins	+	+	+	+	+	+	+	+
13	Lipids	+	+	+	+	-	+	+	+
14	Carbohydrates	-	-	-	+	-	+	-	-

In the present study, the quantitative determination of ash content done in the powder of *Azolla microphylla* showed total ash 48%, acid insoluble ash 50%, and water soluble ash 80%. The estimation of protein, lipid, carbohydrate, energy was carried out protein was present in significant amount (0.25 mg. Carbohydrate (0.18 mg/g) and lipid (0.01mg/g) was also present (Fig: 1).

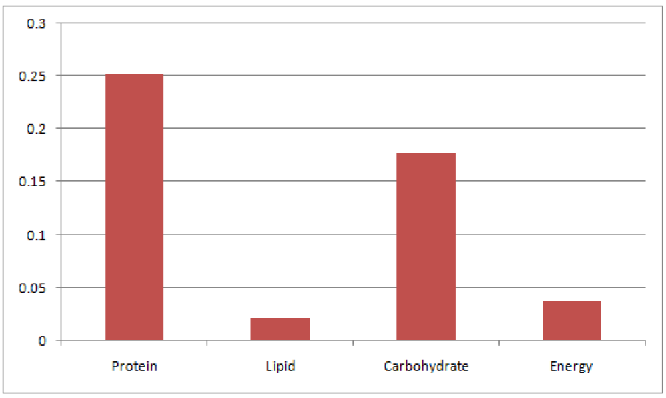

Figure 1. Biochemical parameters of *A.microphylla*

Physicochemical parameters such as total ash (48%), acid insoluble as (50%) and water soluble ash (80%) were also evaluated (Table 2).

Table 2. Physico chemical parameters of *A.microphylla*

S.No.	Particulars	Percentage
1	Total ash	48%
2	Acid-insoluble ash	50%
3	Water-soluble ash	80%

DISCUSSION

The protein composition of *Azolla* is 25-35% on dry weight basis and is easily digested by poultry (Parashuramulu *et al.*, 2013), Van Hove and Lopez (VanHove and Lopez, 1987) noted that the crude protein content of *Azolla* might vary from 13.0 to 34.5%. These variations in the nutrient composition of *Azolla* meal is due to differences in the response of *Azolla* strains to environmental conditions such as temperature, light intensity and soil nutrients which consequently affect their growth morphology and chemical composition (Mila *et al.*, 1996; Ishikura, 1982 and Arai *et al.*, 1998). *Azolla* has a higher crude protein content (ranging from 19 to 30 percent) than most green forage crops and aquatic macrophytes and a rather favourable essential amino acid (EAA) composition for animal nutrition (rich in lysine), it has also attracted the attention of livestock, poultry and fish farmers. Preliminary phytochemical screening revealed the presence of phenolics, tannins, anthraquinone and glycosides confirming the findings of Mithiraja *et al.* (2011). Phenolic compounds are known to have antioxidant properties for plants including Azolla ferns, established under stress conditions (Masood *et al.*, 2008 and Bačkor *et al.*, 2009). Inhibition of bacterial growth by flavonoids has been reported earlier (Mbuh *et al.*, 2007). Environmental conditions play an important role in relation to the content of secondary metabolites and introducing a stress factor to standardize the level and production of these compounds (Kennedy *et al.*, 2011).

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