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Mineral Content and Total Antioxidant Activity of Different Extracts of *Azolla microphylla*

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Abstract: Pteridophytes are reported to have biological and medicinal value since ancient times but a few works have been carried on the wetland pteridophytes. In the present work *Azolla microphylla*, a pteridophyte was selected for the mineral analysis and *in vitro* total antioxidant activity. The whole plant powder was investigated for mineral components by X-ray diffraction analysis. Then the plant powder was undergone extraction process by Soxhlet apparatus using aqueous and hydro-acetone (70:30) as solvent for 8 h. The extract was investigated for the total antioxidant activity by phosphomolybdenum method, a standard procedure for the analysis of polar and non-polar compounds. The results of the mineral analysis showed that the carbon, oxygen, potassium, calcium, sodium, magnesium and silicon were present within the prescribed limits as listed by WHO, FDA and EFSA. No toxic minerals and heavy metals were observed. The antioxidant activity of the two different extracts compared with the standard known ascorbic acid. There was no significant difference ($p < 0.05$) observed between known ascorbic acid and hydro-acetone extract. Significant differences ($p < 0.05$) were observed in both known ascorbic acid with aqueous extract and aqueous extract with hydro-acetone extract. The results of this study concluded that no toxicity was reported in the plant powder. The two extracts studied were having antioxidant activity in a dose dependent response as mainly the hydro-acetone extract showed a significant antioxidant response in it. Hence the *Azolla microphylla* was safe and antioxidant potential wetland/aquatic plant.

Keywords: Pteridophytes, Aqueous, Hydro-acetone extract, Antioxidant, Minerals, *Azolla microphylla*

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Introduction

Pteridophytes are less widely distributed than angiosperms. They produce phytochemical with unique ecological purposes relating to herbivore immune mechanism. They are divided into two monophyletic cell lineages, such as lycophytes

and the ferns. The ferns are a vital phylogenetic linkage between the higher and the lower plants. There are 12,000 species of ferns worldwide (Hui Cao *et al.*, 2017). According to Sushruta and Charaks, certain ferns are prescribed in the texts

of Samhita of Ayurvedic medicine. Present investigation reported that the ferns and allies have ethnobotanical and pharmacological importance. Pteridophytes are known to mankind as medicinal plants for more than 2000 years. Phenolic compounds are the wide spread phytochemicals in pteridophyte. When these plants are in stressful conditions, they synthesize phenolics, by an enzyme phenylalanine ammonia lyase (PAL) which acts as a signaling molecules in the synthesize mechanism of flavonoids. A high total phenolic content (TPC) can partially be described by the presence of the conditions in areas where the ferns are identified (Bandyopadhyay and Dey, 2022). Pharmacological and phytochemical analysis of pteridophytes are poorly understood for the medicinal properties. Pteridophytes are vastly used in India, but there are less studies about the medicinal effects. Hence, this study focused on the *Azolla microphylla*, one of the Pteridophyte available in the southern parts of India, mainly in the delta regions of Tamil Nadu and investigated the mineral and *in-vitro* total antioxidant potential.

Materials and Methods

Plant source:

Azolla microphylla (AM) was collected during October to January (2018-2019) in local farm of delta regions in Nagapattinam District, Tamil Nadu, India. The plant material was washed gently with running tap water, rinsed with distilled water and kept for air drying. Air-dried samples were grounded to a fine powder by mechanical grinder. The fine powder was stored in a dry air tight container to avoid any contamination. The powder thus prepared was used for further studies.

Mineral analysis:

The mineral analysis was determined using X-ray diffraction (XRD) analysis. This data was collected using a PANalytical X'Pert Pro MPD diffractometer in a θ - θ configuration employing Cu K α radiation ($\lambda=1.54 \text{ \AA}$) with a fixed divergence slit size 0.5° and a rotating sample

stage. The samples were scanned between 5° and 100° with an X'Celerator detector. The ground powders were manually frontloaded into a standard circular sample holder. Powdered sample were subjected to an intense X-ray beam and diffracted beam was detected. The peaks obtained were analyzed according to the intensities using Joint Committee on Powder Diffraction Standards data and the peaks were matched with the minerals present in the database (Haritha *et al.*, 2015).

Extraction:

The whole plant powder has been extracted with different solvents i.e., distilled water, hydro-ethanol, hydro-methanol and hydro-acetone in the ratio of 30:70. 20g whole plant powder was extracted with 200 ml of the particular solvents by using Soxhlet apparatus for 8 h. After extraction by Soxhlet apparatus, it was strained by Whatman filter paper and obtained the filtrate, and evaporated to dryness by open-disc evaporation using water bath. The weight of the dried extracts (yield) obtained from different solvents was recorded by using electronic balance. The extract was subjected to total antioxidant activity (Mohammad Reza *et al.*, 2010).

Total antioxidant activity by phosphomolybdenum method:

Various concentrations of Ascorbic acid (standard) and plant compound was taken in the test tubes (20-100 $\mu\text{g/ml}$), 0.5 ml of 0.6 M sulphuric acid, 0.5 ml of 28 mM Mono sodium phosphate and 2 ml of 4 mM ammonium molybdate was added and mixed well. The reaction mixture was incubated at 95°C for 90 min for the color development. The green color mixture was obtained and their absorbance was read at 695 nm. Ascorbic acid was used as the standard (Ganesh *et al.*, 2004; Rashid *et al.*, 2010). Percentage of inhibition was calculated using the following formula:

$$\% \text{ of Inhibition} = \frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \times 100$$

where OD is the optical density.

Statistical analysis:

Experiments were done in triplicates and results are shown as mean $\pm$ SD (standard deviation). Levels of significance and Regression coefficient (R^2) were determined by Student's t test by using Ms-Excel where all the column of treatments was compared with the control.

Results and Discussion

Azolla microphylla an aquatic fern was subjected to mineral analysis by X-Ray diffraction analysis. Plant powder contained carbon (60.03%), oxygen (33.54%), potassium (2.15%), calcium (1.52%), chlorine (1.48%), sodium (0.49%), magnesium (0.48) and silicon (0.32%) (Table 1; Fig. 1). From the results, the carbon was reported in a higher proportion, though it is not a mineral it plays an important role in the growth of plants, as it takes 45-50% of the plants dry weight. According to FDA, the carbons in the direct diet are rare, as it plays a role in the component of bio-molecules, such as carbohydrates, proteins, etc. Approximately 7 to 8 million species of animals including humans need carbon to consume a lot. But the FDA lists omits directly the carbon, nitrogen, hydrogen and oxygen (Mary, 2022). Minerals have healing as well as protective role in diseases condition. Calcium, potassium, phosphorus, and magnesium are macro-nutrients. Potassium is demonstrated to increase iron utilization and is useful for the reducing hypertension.

Potassium and sodium undergo acid-base balance and nerve transmission. The potassium is necessary for maintaining normal water-electrolyte balance of the body and equilibrium osmosis in the cells. It plays role of activation of wide number of enzymes and involved in metabolism of carbohydrate and protein. European Food Safety Authority (EFSA) recommended that daily allowance for potassium must be 3500 mg/day. The calcium (1.52%) is the third main minerals in the powder. Calcium plays a wide range of useful functions in the body, in

order to maintain normal blood calcium level, for adults an average consumption is recommended as 750 mg/day for women and 950 mg/day for men. Calcium is needed for the development and proper functions of the bones, muscles and teeth. Sodium, potassium and calcium also impact heart functions. Calcium increases heart shrinkage. Sodium is next minerals in the proportion of 0.49%. According to the EFSA, the analysis for the total needed for sodium and chloride is under research. According to the WHO, the average intake of sodium for adult should not exceed 2 g/day (Katarzyna *et al.*, 2020).

The magnesium (0.48%) is the other minerals, which is co-factors for many enzymes mainly in energy metabolism. The daily need for magnesium for an adult is 400 mg/day. Lastly silicon is a trace element, the intake of silicon varies with regions, and for example the consumption of silicon is 140-204 mg/day for Indians and Chinese. The literature search set the intake of 20-50 mg per day. It plays a vital role in the physiological and metabolic functions in plants and humans. Silicon is responsible for the metabolism of connective tissue, biosynthesis of collagen, glycosaminoglycan, which is required for the formation of bone. It is also involved in the function of polyhydroxylase, a chief enzyme involved in the formation of collagen, elastane, cartilage and connective tissues (Anna and Franciszek, 2020).

Antioxidant activity by phosphomolybdenum method:

The whole plant powder of *Azolla microphylla* was air dried and subjected to extraction by Soxhlet method by two solvents with different polarities, water (a high polar solvent) and hydro-acetone (High polarity (70): (30) medium polarity). Then the two extracts were endured for total antioxidant activity by phosphomolybdenum method. The method worked on the principle of the reduction of phosphomolybdate ion in the presence of an antioxidant results in the formation of green color complex of phosphate/moV complex. The antioxidant activity

Table 1: Mineral content in the whole plant powder of *Azolla microphylla*

Mineral Content	Series	Weight (%)	Atomic (%)
Carbon	K-Series	60.03	68.65
Oxygen	K-Series	33.54	28.79
Potassium	K-Series	2.15	0.75
Calcium	K-Series	1.52	0.52
Chlorine	K-Series	1.48	0.57
Sodium	K-Series	0.49	0.29
Magnesium	K-Series	0.48	0.27
Silicon	K-Series	0.32	0.15

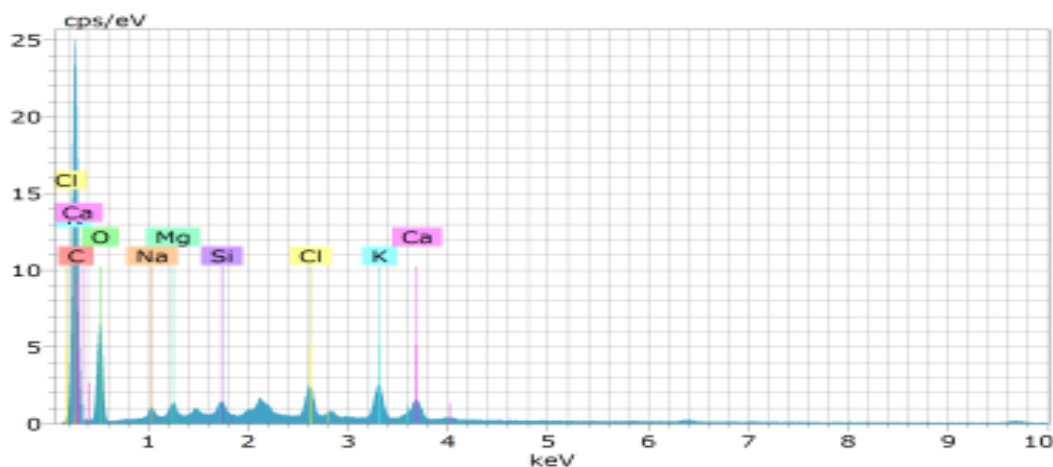


Fig. 1: Mineral content in the whole plant powder of *Azolla microphylla* X axis-Kev; Y axis-cps/ev (Different colors were heightened for different mineral content).

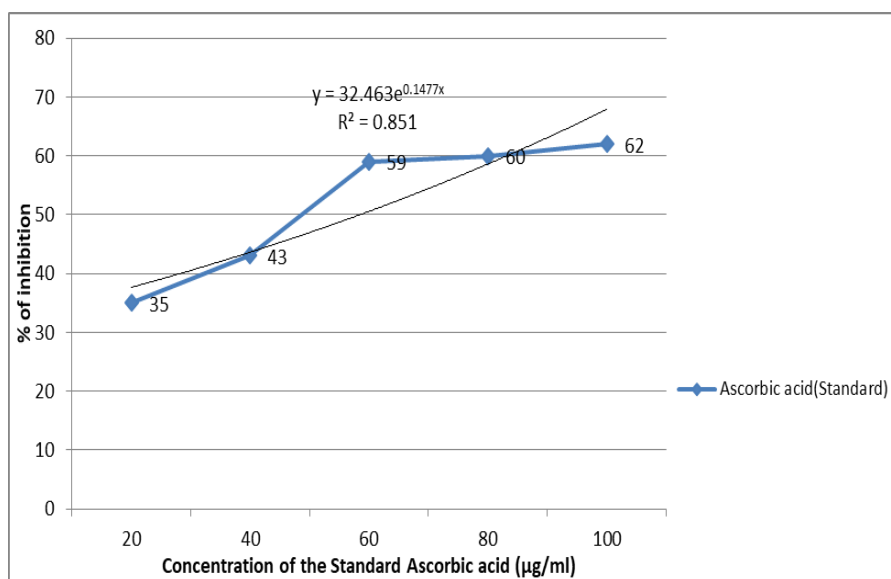


Fig. 2: Percentage inhibition of the standard Ascorbic acid. R=regression coefficient, n=3. Values are expressed in mean $\pm$ SD.

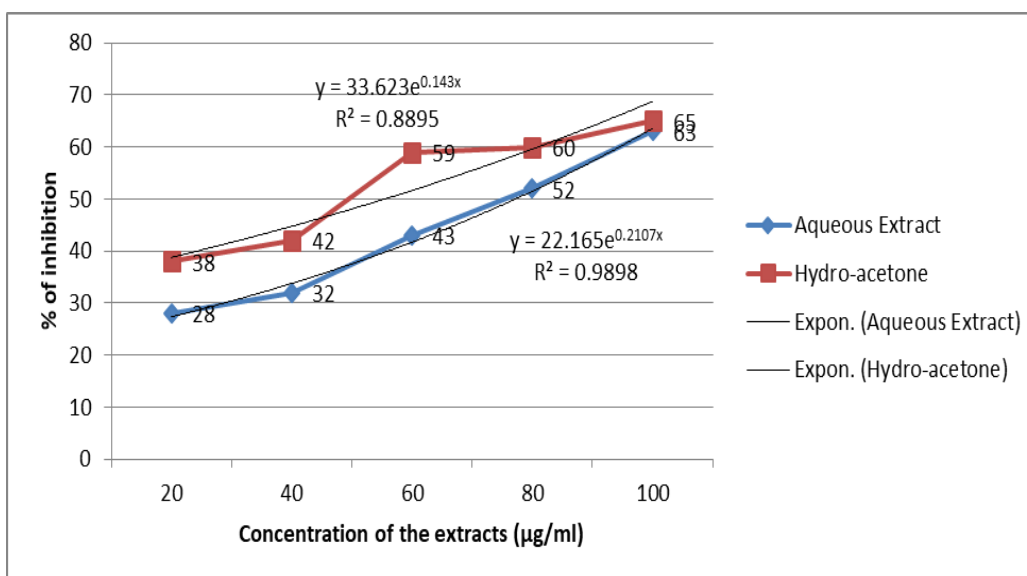


Fig. 3: Percentage inhibition of the Different extracts: R=regression coefficient, n=3. Values are expressed in mean $\pm$ SD.

of Vitamin C (Ascorbic acid) was studied previously, hence used as a positive control. The two different extracts were compared and the results are shown in Figure 2 and 3. From the results, it was clear that, the antioxidant activity was dose dependent. The total antioxidant potential method evaluated the increase in phenolic compounds reducing the capacity of the antioxidant (Phatak and Hendre, 2014). The exponential regression coefficient of the standard ascorbic acid was $R^2 = 0.85$, whereas for the aqueous extract and hydro-acetone extracts $R^2 = 0.989$ and $R^2 = 0.889$, respectively. The results for the comparison, for standard ascorbic acid with aqueous extract showed a significant difference between the two ($p > 0.05$) with the value of 2.19. No significant difference ($p < 0.05$) was noticed in between the standard ascorbic acid and the hydro-acetone extract with the t value of 0.29. By comparing the two extracts there was a significant difference ($p > 0.05$) in between the aqueous and hydro-acetone extract with the t value of 2.42. The known ascorbic acid (vitamin C) acts as a natural antioxidant in scavenging the body from the formation of free radicals. As free radicals cause many clinical

effects to the body and leading to diseased conditions such as diabetes, Alzheimer's disease, inflammation and cancer. The hydro-acetone extract exerts a similar scavenging activity against free radicals, as an antioxidant against ascorbic acid. In the biological system, the reactive oxygen species are generated and they cause damage to the tissues and the molecules leading to different diseases, mainly degenerative diseases and extensive lysis. The synthetic drugs protect against oxidative damage, but produce adverse reactions.

Conclusion

The minerals present in the plant powder were within the permissible limit or in low quantity. Further, the extracts of the *Azolla microphylla* showed an antioxidant activity similar to the known antioxidant ascorbic acid. The hydro-acetone showed a significant activity when compared with the aqueous extract, although the antioxidant activity was dose dependent. In future, the *Azolla microphylla*, needs to be further investigated against human diseases, because it is safe for human consumption and it is proved as an antioxidant.

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