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PHYSICO CHEMICAL AND ELEMENTAL ANALYS IS OF THE WHOLE PLANT POWDER OF *AZOLLA MICROPHYLLA*

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ABSTRACT: Introduction: Anabaena azollae (AM) is used as a biofertilizer feed for cattle; much research is developing toward identifying the elements and their role in food supplements for human consumption. **Objective:** The study was to characterize the whole plant powder of the *A. microphylla* concerning physicochemical and elemental analysis. **Material and Methods:** The whole plant powder was subjected to physicochemical analysis such as total ash content, acid insoluble ash content, and moisture content by standard procedure. Further, it was analyzed for elements through SEM and XRD studies. **Results:** The results revealed that the total ash content, acid insoluble ash content and moisture content were within limits. The morphological feature of the *A. microphylla* whole plant powder showed a compact structure. The XRD showed that the elements such as carbon, oxygen, potassium, calcium, chlorine, sodium, magnesium, and silicon were present in descending order and within the normal limits. **Conclusion:** The presence of the inorganic elements in the whole plant powder of *A. microphylla* was within limits for the plants' standardization. Hence, the aquatic plant serves as the cure for aliments in the further aspects of drug discovery.

INTRODUCTION: India is considered a hot spot region for plant diversity, and about one-fifth of the Indian plant are found to have medicinal properties [1]. It is estimated that about 25,000 plants were used in effective plant formulations and in traditional medicine preparations, especially in rural communities of India [2]. In the Indian system, a large number of medicinal plants have been used for many centuries for treating various diseases. Medicinal plants have been used as remedies for human diseases because of their chemical contents of therapeutic value.

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As of record, around 20,000 plant species are used for medicinal purposes across the globe and around 70 % are from the Indian subcontinent [3]. One-fourth of the world population, *i.e.*, 1.42 billion people, depend on traditional medicines, particularly plant drugs, for curing ailments [4]. Herbal medicines are a promising choice over modern synthetic drugs. They show minimum/no side effects and are considered to be safe. Generally, herbal formulations involve using fresh or dried plant parts [5].

The curing and prevention property of medicinal plants depends on their chemical composition. Various macro and trace elements play a vital role in building up and restoring the health of the human body [6]. Trace elements have important roles in combating a variety of human ailments and diseases which was observed by the study of elements with respect to many indigenous medicinal plants.

These micronutrients are essential and required daily by the body in trace amounts to function properly. Micronutrients can be classified as macroelements and trace elements. The macro elements include phosphorous, chloride, calcium, sodium, magnesium, iron, and potassium. The trace elements include boron, cobalt, copper, sulfur, chromium, fluoride, iodine, selenium, zinc, manganese, and molybdenum [7].

Floating aquatic macrophytes are plants that float on the surface of the water body, generally not dependent on the soil or water depth. Fast-growing free-floating aquatic fern Azolla species is distributed in tropic and temperate freshwater. The genus Azolla was discovered by J. B. L, a mark as early as 1783, belongs to the *Salvinaceae* family of the order Salviniales [8]. Azolla is an aquatic pteridophyte that lives in a symbiotic relationship with the blue-green algae, Anabaena Azollae (AM). Besides being used as a biofertilizer, much research interest is developed towards the identification of the medicinal values of Azolla species. The phytochemical investigation of *Azolla microphylla* shows that tannins, phenols, sugar, anthroquinone glycosides and steroids were present [9]. The aquatic pteridophyte *Azolla microphylla* changes the color of the fonds with respect to the season.

The diversity of the Indian subcontinent with respect to environment regimes such as light intensity and temperature and soil nature will also help in the production of the metabolites. The level of the elements in the plants varies by the characteristics of the soil and also environmental conditions [6, 11]. Hence the present study was carried on the physicochemical and elemental analysis of the *Azolla microphylla* collected from the delta region of Tamil Nadu.

MATERIALS AND METHODS:

Collection of the Plant Material: For the present study, the whole plant of *Azolla Microphylla* (AM) was collected from October to January (2018-2019) in and around Thanjavur District. 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. The powder was kept in small plastic bags with proper labeling for further use. The plant was authenticated by a botanist (Voucher No: DGGACBOT HR-10), Department of botany, D. G. Govt Arts College (W), Mayiladuthurai, Nagapattinam (DT), Tamil Nadu, India.

CHEMICALS: All chemicals used in this study were of analytical grade obtained from Sree Sabari Scientifics, Mayiladuthurai, Nagapattinam (DT), Tamil Nadu, and India.

Physicochemical Analysis: The whole plant powder of *A. microphylla* was carried out for physicochemical analysis by standard procedure [4].

Estimation of Total Ash: 1 g of the plant powder under examination was taken in the silica crucible, dried at 100-105 ºC for 1 h, and ignited to constant weight in a muffle furnace at 600 ± 25 ºC. After prolonged ignition, carbon-free ash obtained from the charred mass was washed with hot water, and the residue was washed on an ash-less filter paper; the residue and filter paper was incinerated until the ash was white or nearly white; the filtrate was added to the dish and evaporated to dryness and ignited at a temperature not exceeding 450 ºC. The percentage of ash was calculated on the dried drug basis, as follows

$$\% \text{ Total ash value} = \text{Weight of ash}/\text{Weight of drug} \times 100$$

Estimation of Acid-insoluble Ash: The ash (total ash) was boiled with 25 ml of 2M hydrochloric acid for 5 min; the insoluble matter was collected in an ash-less filter paper, washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was collected on a dried drug basis. The percentage of ash was calculated on the dried drug basis, as follows

$$\% \text{ Acid-insoluble ash value} = \text{Weight of ash}/\text{Weight of drug} \times 100$$

Estimation of Moisture Content: Moist sample (5 g) was weighed immediately and recorded as 'wet' weight of sample'. The wet sample was dried at a temperature not exceeding 115 ºC using a hot air oven. The sample was allowed to be cooled and to be weighed again after 3 h and was recorded as the 'dry weight of sample', successively.

The amount of moisture was calculated using the following formula:

$$\% \text{ Moisture content (Wb)} = Ww - Wd/Ww \times 100$$

Ww: wet weight of sample; Wd: dry weight of sample

Sample Preparation for Scanning electron Microscope: The dried samples were grounded and fixed on an adhesive tape and then coated with a thin gold layer by a sputter coater. A high range of X-ray beams was made to fall under a high vacuum mode to perform the scanning of the specimen. The voltage and magnifications in the test setup were altered until a more refined picture was observed. Scanning electron microscope (SEM) images and compounds present were observed and identified based on their shapes and colors compared to standards of those particular materials [12].

Scanning Electron Microscopy Analysis: A SEM is an electron microscope used to analyze the sample images with a focused beam of electrons. The electrons interact with atoms in the sample and produce various signals that can be detected and surface topography and composition information can also be obtained. In this study, microstructural characterization was carried out by using field emission SEM (FESEM, Carl Zeiss and Supra 40) [12].

X-ray Diffraction Analysis: The elemental analysis was determined using X-ray diffraction (XRD) analysis. This data was collected using a PAN analytical X' Pert Pro MPD diffractometer in a θ-θ configuration employing Cu Kα radiation (λ=1.54 Å) 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. A powdered mix sample was subjected to an intense X-ray beam, and the 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 [12].

RESULTS:

TABLE 1: THE PHYSICOCHEMICAL ANALYSIS OF WHOLE PLANT POWDER OF *AZOLLA MICROPHYLLA*

Chemical parameters	Contents
Color	Brown
Structure	Crystalline
Odor	Pleasant
Nature	Hygroscopic
Total Ash content	13.0 (g %)
Acid Insoluble ash	10.3(g %)
Moisture content	3.0(g %)

(g%-gram percentage)

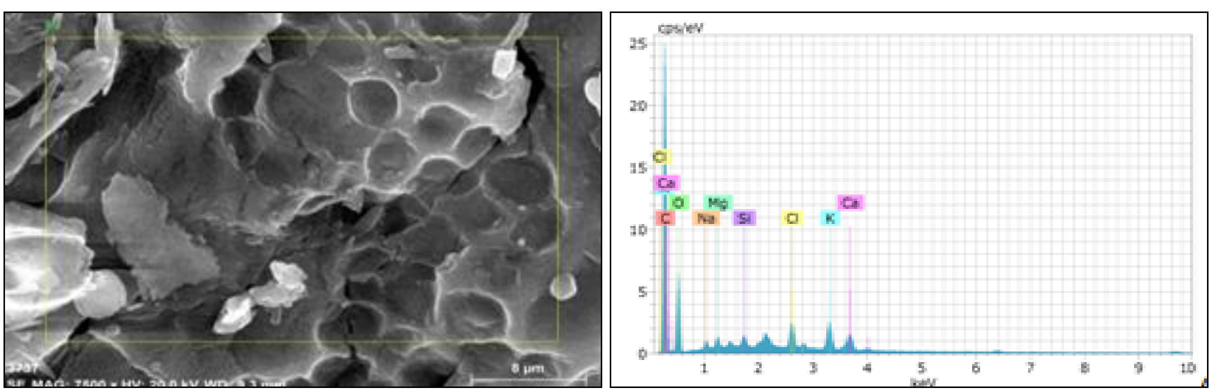

FIG. 1: ELEMENTAL ANALYSIS OF AZOLLA MICROPHYLLA WHOLE PLANT POWDER, (A) SHOWN THAT THE AREA FROM WHERE SEM ANALYSIS HAS BEEN TAKEN AND (B) THE AREA WHERE THE ENERGY-DISPERSIVE X-RAY SPECTROSCOPIC SPECTRUM ANALYSIS THE SAMPLE

Fig. 1A: Scanning Emission Microscope (SEM) analysis was carried out in the whole plant of *A. microphylla* for morphological characteristics. The morphology of the powdered sample was observed with an accelerated voltage of 20.0 kV.

The powdered plant sample was crystal structure with compact nature in an uneven surface **Fig. 1A.** It was not porous. Each crystal was hexagonal in a structure of width 9.3 mm and a diameter of 8 µm.

TABLE 2: ELEMENTAL ANALYSIS OF *AZOLLA MICROPHYLLA*

Element	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

(%- Percentage)

Fig. 1B and **Table 2** The X-Ray Diffraction (XRD) analysis was carried out in the whole plant of powdered *A. microphylla* for elemental composition. The slight shift in the peak positions indicated the presence of strain in the crystal structure **Fig. 1B**. The whole plant powder of *A. microphylla* includes various distinct chemical elements with different weights. Therefore, the weight percentage of different elements such as Carbon, Oxygen, Potassium, Calcium, Chlorine, Sodium, Magnesium, and Silicon was analyzed. **Fig. 1A** depicts the area where the XRD was analyzed for the elements present in the plant powder. The XRD spectrum of element analysis was tabulated in the above table. Carbon showed the highest weight of 60.03% among the different elements in the plant powder, followed by oxygen (33.54%), potassium (2.15%), calcium (1.52%), Chlorine (1.48%) while Sodium, Magnesium, and silicon were present in trace quantities. From the above presentation, it was obvious that most of these plants accumulate essential elements from the soil for the plants, humans, and animals.

DISCUSSION: Plants still constitute one of the major sources of drugs in modern and traditional medicine worldwide. It has been used to cure diseases since antiquity. In the study, the physicochemical and the elemental analysis were carried out in the whole plant of *A. microphylla* in powder form. The physical characteristic of the plant powder of *A. microphylla* includes a deep brown colored crystalline structure with characteristic cod or hygroscopic. The total ash is particularly important in evaluating the purity of drugs, *i.e.*, the presence or absence of foreign inorganic matter such as metallic salts and/or silica [13]. So that a care should be needed in preparing the plant for medicinal use. The ash content obtained was 13% (normal limits < 15%), which implies that the powder form of the whole plant of *A. microphylla* includes low inorganic materials. The acid-insoluble ash values of the herbal sample indicate the presence of natural impurities like carbonate, oxalate, and silicate.

At 105 ºC, the moisture content was calculated as 3.0% (normal limits > 6% at 105 ºC). Moisture content provides information on drying and the presence of excess water in the crude plant powder. It is also important because an excessive amount of water in a plant material induces microbial reproduction and it causes deterioration on the presence of water content (hydrolysis) [14]. Medicinal plant materials should avoid any visible signs of contamination by moulds which may produce toxins or insects and other animals which may produce contamination in the form of their feces (excreta) [15].

Then the whole plant powder of *A. microphylla* was analyzed for morphology by using SEM, which showed a hexagonal structure **Fig. 1A**, supporting information). The surface area was uneven and the area was further subjected to elemental analysis through X-Ray Diffraction studies **Fig. 1B** and **Table 2**, supporting information). The results revealed that the elements carbon, oxygen, potassium, calcium, chlorine, sodium, magnesium and silicon were present in the *A. microphylla* whole plant powder in descending order.

Carbon showed the highest weight of 60.03% among the different elements in the whole powder, followed by oxygen (33.54%), while potassium, calcium, chlorine, sodium, magnesium and silicon were present in trace quantities. The human body requires more than 20 elements for the normal function of the body system. The various elements are required in amounts ranging from 50 µg to 18 mg per day for the body's functioning. Deficiencies in such essential nutrients lead to diseases including bone and muscle dysfunction, metabolic disorders, and nervous disturbances. The presence of elements causestoxicityto the human body, even at a low amount. Plants absorb various elements present in the soil and water by the root hairs, and the absorption rate is variable for different elements. Most significantly the heavy metals like Cd, thallium, and zinc have higher transfer coefficients and are readily taken up by plants. In contrast, Pb, Cr, co, and Cu have a lower transfer coefficient to plants from the soil as they remain stably bound to the soil system [16]. The concentration of heavy metal content within the crude plant sample is necessary due to the variability of habitat selection. Very little information is available about the potential influence of metals on the pharmacological activity of natural drugs obtained from medicinal plants from varied locations, especially from contaminated sites. Medicinal plants are used in folk herbal preparations and as food supplements. Many of them are utilized as vegetables and other food preparations like condiments. Therefore, it is very necessary to assess the heavy metals level in medicinal plants before they are utilized as medicines and condiments as these metals have lethal physiological effects in a higher threshold level [17]. In their guidelines, World Health Organization/Food and Agriculture Organization also has put forward this critical issue and recommended the determination of heavy metal content in herbal medicines [18].

The carbon is in the form of carbonate and bicarbonate ions, and it plays an important role in pH regulation and maintains acid-base balance in the human body [19]. It indicates that the aquatic fern is a source of hydrocarbons which are the building blocks of many primary and secondary metabolites. A higher carbon to nitrogen ratio in organic material is indicative of being more carbonaceous in the form of carbohydrates and proteins, increasing the strength of the cell wall [20].

Potassium was present in the percentage of 2.5 g in the whole plant powder of *A. microphylla*, this value was within limits. It is an important intracellular cation element and helps maintain osmotic pressure and pH equilibrium. It also plays an important role in maintaining normal glucose with the release of insulin from the pancreas, i.e., from the beta cells of Langerhans. It is accumulated within human cells by the action of the Na+ K+ ATPase (sodium pump) and it is an activator of some enzymes, particularly coenzymes, for normal growth and muscle function. It is involved in the metabolism of protein and carbohydrates. Potassium deficiency causes nervous disorder, diabetes, and poor muscular control resulting in paralysis [9].

Calcium was present in the percentage of 1.5 g in the whole plant powder of *A. microphylla*; this value was within limits. It plays an important role in the bone function and decreases blood glucose levels utilizing insulin [9]. It helps in preventing and curing all bone-related disorders. It helps to repair worn-out cells; strong teethe in humans, synthesis of RBCs [21]. Ca is an essential mineral for healthy bones, teeth, blood muscles, and nerves. It is needed for the absorption of vitamin B, for the synthesis of the neurotransmitter acetylcholine, and for the activation of enzymes such as the pancreatic lipase [22]. Chlorine was present in the percentage of 1.48 g in the whole plant powder of *A. microphylla*; this value was within limits. It acts as an anion of the extracellular, connective tissue, lymph, bone, and cartilage.

Magnesium was present in the percentage of 1.48g in the whole plant powder of *A. microphylla*, this value was within limits. It also plays an important role in regulating the muscular activity of cardiac rhythm and acts as an important cofactor in converting blood glucose into energy [12]. It plays an important role in the metabolism of cholesterol and heart disease. It regularizes electrical potential in nerves and nerve membranes. It improves insulin sensitivity and protects against diabetes and diabetic complications. Magnesium lactate inhibits histidine decarboxylase and prevents the formation of histamine from the amino acid histidine [13]. Ca+2 and Mg+2 are present in exchangeable amounts and act as binding agents to fuse the cell walls together [14].

Silicon was present in the percentage of 0.32 g in the whole plant powder of *A. microphylla*; this value was within limits. It plays an important role in strengthening connective tissues and bones, useful in caring for nails, hairs, and glowing skin in human health.

It plays a crucial role in preventing diseases such as atherosclerosis, insomnia, skin disorders, and tuberculosis. It is considered by the association of American Plant Food Control Officials for elevation to the status of a plant beneficial substance as strengthened cell walls improve plant strength, health, and productivity. It is considered a major element to prevent the arteries and veins hardening [15]. Silicon is mainly found in skin and cartilage but is also present in other tissues which contain glycosaminoglycans (heparin, hyaluronic acid) and collagen.

Elements in the plant depend on the composition of the soil, water, and fertilizers used, and plants' permissibility, selectivity, and absorbability for the uptake of the elements [16]. From this observation, the presence of the elements and the variations in the concentration of the elements are attributed to the nature of the plant *A. microphylla* and its surroundings in the delta region.

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CONFLICTS AND INTEREST: Nil

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