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Evaluation of the Phytochemical Profile and Pharmacognostic Indicator of Crude Whole Plant Powder of *Azolla microphylla* by Fluorescence Analysis with Different Chemical Reagents

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Abstract: *Azolla microphylla* is an aquatic fern used as a biofertilizer in agricultural fields. It helps in nitrogen fixation with a close symbiotic relationship with the blue-green algae *Anabanea*. The aim of the study was to evaluate the phytochemical profile and pharmacognostic indicators of crude whole plant powder of *Azolla microphylla* by fluorescence analysis with different chemical reagents. The phytochemicals, such as alkaloids, cardiac glycosides, saponins, phenols, tannins, flavonoids, and diterpenes, were present in both the hydro-ethanol and the hydro-methanol extract of *A. microphylla,* while the FTIR spectrum revealed the presence of functional groups. The fluorescence results showed the presence of certain phytochemicals, such as flavones, coumarins, and phenols, in the plant powder and different color shades showed that the plant powder may have a new phytocompound. This study brings a new dimension to the documentation of new phytochemicals present in the whole plant of *Azolla microphylla,* which will play a novel role in the pharma industry in future research.

Keywords: Phytochemical, Fluorescence analysis, *Azolla microphylla,* FTIR

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Introduction

Since years, plants serve to human as a natural source for curing various ailments and pathological/clinical conditions. Several studies focus on the plant source and recent enormous evidences suggested the huge potential of plants used in various systems of traditional medicines. World Health Organization concentrates on the National health care programes as the plant medicines are easily available at minimum cost, safe, and people rely on them. It is an essential factor to check and assure the nature and quality of the crude drugs used in the authentication and identification of the plant medicines (Bhutia, 2020). For the confirmation of the identification

and authentication the quality and purity of the raw drugs, the best and reliable criteria are pharmacognostical testing. Complete information about the raw drugs can be produced by simple and reliable method by pharmacognosy. Fluorescence is an important phenomenon that variety of chemical compounds in the plant powder exposited. Many natural chemical compounds do not glow in day light, but under UV light they emit fluorescence. As a results, several crude drugs are frequently evaluated qualatitatively, which is an essential pharmacognostical indicator (Jeevitha *et al*., 2021). A correlation exits between a chemical compound present in the raw drugs and their fluorescent behavoiur under different conditions.

Azolla, the genus is defined as a heterosporus leptosporangiate fern from aquatic and semi-aquatic characteristics. It is assigned to the monogenic family, Azollaceae. It is small aquatic plant which appears to float on the water surface. It is a unique freshwater fern, that is one of the fastest growing plants on the Earth due to its symbiotic relationship with a cyanobacteria (a blue-green algae) called *Anabanea* (Azolla foundation.org). This research work is carried out on the plant *Azolla microphylla*, (one of the species of the *Azolla* family) for the first time. This work gives a clue about the whole plant powder that are suitable to act as a template for the identification and authentication of new drug materials from the aquatic plant.

Materials and Methods

Plant material:

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 (Pantastico *et al*., 1986).

Phytochemical screening:

Chemical tests were carried out on the alcoholic extracts using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Fluorescence Analysis:

The fluorescence analysis of the powdered samples of *Azolla microphylla* with various solvents and chemical reagents was performed as described by Kokoshi *et al.* (1958) and Gupta *et al.* (2006). The behavior of the sample after treatment with different chemical reagents and solutions was observed under visible light/day light; short (254 nm) and long (365 nm) wavelength ultra violet light and the observations were recorded.

Results and Discussion

Azolla microphylla extracts possess phytochemicals (Table 1), which is evidenced by remarkable color changes. Alkaloids, cardiac glycosides, saponins, phenols, tannins, flavonoids, and diterpenes were present in both the hydro-ethanol extract and the hydro-methanol extract of *A. microphylla*. Flavonoids, alkaloids, and phenols were the most abundant classes of compounds in the majority of the screened plants (Dubale *et al.,* 2023). Plant products are an important part of the human diet and contain biologically active substances, including antioxidant compounds. Approximately 50000 metabolites have been elucidated in plants, and it is predicted that the final number will exceed 200000. Secondary metabolites such as flavonoids and phenolics have antioxidative, anticarcinogenic, anti-hypertension, anti-inflammatory, antimicrobial, immunostimulant, and cholesterol-lowering properties (Velavan, 2015). Shunmuga Vadivu and Velavan (2020) reported that the enzyme inhibitory effect may be attributed to the presence of secondary metabolites in plants. The literature supports evidence that *Azolla*

Table 1: Phytochemical analysis of *A. microphylla* extracts

Phytochemicals	Name of the test	Hydro-ethanol extract	Hydro-methanol extract
Alkaloids	Mayers test	+	+
	Wagner's test	+	+
	Hager's test	+	+
Amino acids	Ninhydrin test	+	+
Proteins	Xanthoprotein test	+	+
Carbohydrates	Molisch s test	-	-
Reducing sugar	Benedicts test	+	-
Glycosides	Borntrager's test	-	+
Cardiac glycosides	Legal's test	+	+
Saponins	Froth test,	+	+
	Foam test	+	+
	Salkowskis test	-	+
Phytosterols	Liebermann Burchard test	-	+
Phenol	Ferric chloride test	+	+
Tannins	Gelatin test	+	+
Flavonoids	Alkaline reagent test	+	+
	Lead acetate test	+	+
Diterpenes	Copper acetate test	+	+

+ Present; -Absent

microphylla phytochemicals are involved in various biological properties.

The FTIR is proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. The FTIR spectrum of the plant extract is given in Figure 1. The FTIR analysis of plant extract gave broad peak at 3387.33 cm^{-1} which indicated the presence of phenolic OH stretching. Peaks from 2901.94 to 2971.25 cm^{-1}represent alkane C–H stretching. It showed strong peaks at 1710.37 cm^{-1} represent C=C stretch which indicated the presence of alkenes and C-C stretch (in-ring) attributed to benzene ring present (aromatic ring) whereas 1049.70 to 1159.25 shows –C-O stretch which indicates alcohols. Our finding of the flavonoids is in agreement of many researchers as their investigation showed that flavonoids compounds have major functional groups –OH group, -OH bending, C=O group, C-H stretch and C-H bending groups (Maria *et al*., 2018). The FTIR spectrum revealed the presence of functional groups involved in metal reducing and capping agents, leading to the formation of metallic nanoparticles (Karnan *et al.,* 2023 a, b). Fluorescence analysis is an efficient, responsive and precise method for the identification of drugs, i.e. for the determination of various compounds in a short period of time compared to several time – consuming dilutions steps involved in the analysis of pharmaceutical samples (Singleton *et al.,* 1965; Lindley, 1998). Fluorescent color shows specific nature for particular compound. The different plant source show various coloration when subjected to different chemical reagents during testing. Hence, the study concentrates on the fluorescence analysis of the whole plant powder of *A. microphylla*. The samples were added to different chemical reagents with varying nature (day light and UV light) to observe the color reactions. Hence, the raw powder was assessed qualitatively in this way and the result is illustrated in Table 2. The powder was exposed under visible day light and UV light and the colors observed in the analysis were dark brown, light brown, dark green, light green, light pale yellow, dark yellow, orange,

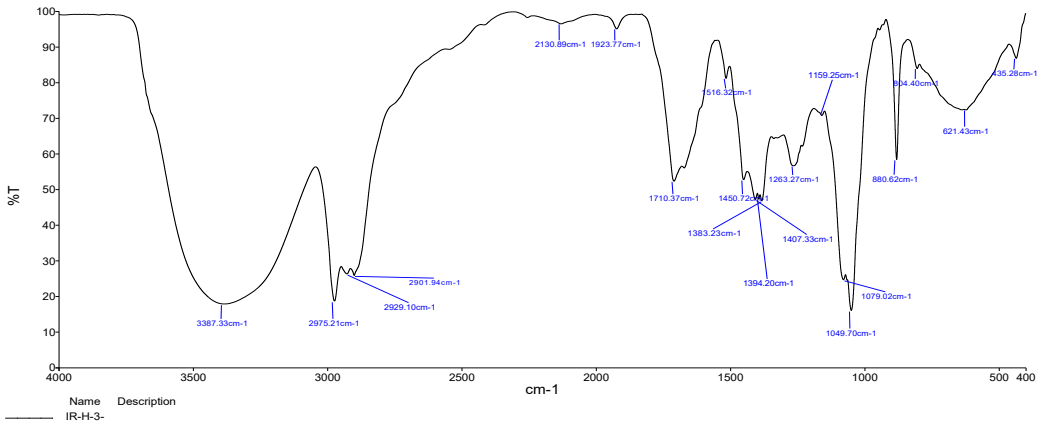

Fig. 2: FTIR spectrum of *Azolla microphylla* Hydro-methanol extract.

Table 2: Fluorescence analysis of whole plant crude powder of *A. microphylla*

S. No.	Sample	Visible light/Day light	UV fluorescence	
			254nm	365nm
1	Powder as such	Brown	Brown	Brown
2	Powder + 1N Aqueous NaOH	Dark brown	Dark brown	Dark Brown
3	Powder + 1N Alcoholic NaOH	Green	Dark brown	Light cream
4	Powder+ Con.H_2SO_4	Dark brown	Dark brown	Light brown
5	Powder+ 50% H_2SO_4	Light brown	Dark brown	Light brown
6	Powder+ Con.HCL	Pale yellow	Dark brown	Dark brown
7	Powder+ Con.HNO_3	Orange	Light cream	Light cream
8	Powder+ $Fecl_3$	Greenish brown	Dark brown	Dark brown
9	Powder+ Benzene	Golden yellow	Dark brown	Dark brown
10	Powder+ Ammonia	Reddish brown	Dark brown	Dark brown
11	Powder+ Acetic acid	Golden yellow	Dark brown	Dark brown
12	Powder+ chloroform	Light green	Dark brown	Dark brown
13	Powder+ 2-Propanol	Light green	Light green	Light green
14	Powder+ Diethyl ether	Dark green	Dark green	Dark green
15	Powder+ Picric acid	Dark yellow	Light yellow	Light yellow
16	Powder+ Acetone	Light green	Dark green	Dark green
17	Powder+ water	Light brown	Light brown	Light brown
18	Powder+ Methanol	Dark green	Dark green	Dark green

greenish brown, reddish brown and golden yellow. Light cream color (colorless) was exhibited in conc. HNO_3 in UV light (254 and 365 nm). 1 N alcoholic NaOH also exhibited light cream color (colorless) in the UV light (365 nm) itself. Some constituents show fluorescence in the visible range in day light. The UV light produces fluorescence in many natural products which do not visibly fluorescence in day light. If substance themselves are not fluorescent, they may often be converted into fluorescent or decomposition products/breakdown products by exposing to the chemical reagents (Majid *et al.,* 2021). Under short UV light, coumarins, especially hydroxyl amino acid derivative appears yellowish green in alkaline conditions, where flavanones which appear to be light yellow in aqueous condition, but under UV light, it appears to be bright yellow under basic conditions.

Phytosterols when treated with 50% H_2SO_4 appear to be green under UV light. Sapogenin and terpenoids showed a yellow green fluorescence under short UV light (Harborne, 1976). Quinine,

aconitin, berberin and emetin show specific colors of fluorescence. Least fluorescence is shown by fixed oils and fats (Singh *et al*., 2004). Phenolic group (-OH) exhibited brilliant green when treated with $FeCl_3$ under UV light (Jeevitha *et al*., 2021). At varied wavelength used, crude powder of plant showed disparate fluorescent colors due to the presence of the different chemical constituents in the plant material (Gupta, 2006). So, the results of the study answered that a tie-up was found between a compound present in the raw powder and their fluorescent behavior under different conditions which was expressed in coloration such as light and dark brown and different shades of brown such as reddish brown, greenish brown under day and UV light in different chemical nature.

Conclusion

This study brings a new dimension to the documentation of phytochemicals present in the whole plant of *Azolla microphylla*. This identification of the plant material brings control to the quality of the plant material, which suggests this aquatic plant as a medicine or natural product, which will play a novel role in the pharma industry in future research.

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