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Research Article

PHYSICOCHEMICAL AND HEAVY METAL ANALYSIS OF THE LEAF, STEM, AND FLOWER EXTRACTS OF *SPHENOCLEA ZEYLANICA*

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ABSTRACT

Objective: The aim was to identify and elucidate the physicochemical constituents in the different aerial parts of *Sphenoclea zeylanica.*

Methods: In this study, physicochemical parameters, fluorescence, and heavy metals of methanol extract of leaves, stem, and flower of *S. zeylanica* were conducted.

Results: It was seen that the properties and the results observed were significant and hence, the plant *S. zeylanica* can be used to formulate drugs to cure various diseases if the bioactive components are analyzed.

Conclusions: Concentration of heavy metals differed for different parts of the plant *S. zeylanica*. Amounts of heavy metals detected were within permissible limits, and thus the plant can be used for the formulation of herbal remedies.

Keywords: *Sphenoclea zeylanica* physicochemical parameters, Florescence, Heavy metals analysis.

INTRODUCTION

Plants are the principal source of pharmaceutical agents used in traditional medicine [1]. The first step in the identification and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies which are essential for any phytopharmaceutical products used for standard formulation [2]. Popular plant species have been used to isolate and extract biologically active compounds. At present, on the basis of traditional uses, many plants are evaluated for drug development and discovery of newer drug molecules.

Secondary metabolites which are complex chemical substances of different compositions are responsible for curative properties of medicinal plants [3]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [4]. Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. The basis of pharmacognosy standardization, authentication, and study of natural drugs.

Recently, the importance of pharmacognosy has been widely recognized. One of the parameters of pharmacognostic study is identifying adulteration in dry powder form. This becomes necessary because the morphological identity is lost and is easily prone to adulteration once the plant is dried and made into powder form. Pharmacognostic studies help and prevent adulterations by ensuring plant identity and by laying down standardization parameters. These studies help in authentication of the plants. Furthermore, the reproducible quality of herbal products is ensured, which will lead to safety and efficacy of natural products [5].

In the physicochemical analysis, the parameters studied are moisture content, loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values, petroleum ether soluble extractive value, ethyl acetate soluble extractive value, acetone soluble extractive value, etc. Ash value content can be used to be determine the quality and purity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate, and silicate.

Water soluble ash can be used to estimate the amount of inorganic compound present in drugs while the acid insoluble ash includes mainly silica and indicate contamination with the earthy material. Minimal moisture content of drugs is crucial to discourage the growth of bacteria, yeast, or fungi during storage. The amount of the active constituents in a given amount of plant material when extracted with a particular solvent is determined by the estimation of extractive values. A solution containing different phytoconstituents is obtained by the extractions of any crude drug with a particular solvent. Whether the crude drug is exhausted or not is indicated by the compositions of these phytoconstituents which depend on the nature of the drug and the solvent used [6].

The proper functioning of vital organs in the body requires heavy metals as micronutrients. Their assimilation and accumulation in plants are obvious as a result of geo-climatic conditions, and environmental pollution heavy metals are widespread in soil. Heavy metals are discharged into the environment along with other pollutants, through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning, and pesticides used in agriculture [7]. These metals from the environment through air and water are taken up by human beings, animals, and plants. Heavy metals accumulate in both plants and human organs. The sources for micronutrients for man are plants and animals, therefore, it becomes necessary to monitor the levels in biological materials that are required by man for both dietary and medicinal purposes. This is because deficiency or excess of micronutrients can be factors of disease generation.

Recently, the toxicity of trace metals on human health and the environment have gained considerable attention. Plants are the main link in the transfer of heavy metals from the contaminated soil to humans. Damaging effects on humans even at very low concentrations will be seen since heavy metals have low excretion rates through the kidney. Metals such as zinc, copper, iron, manganese, and chromium are essential nutrients. An increase in their intake above certain limits can become toxic to health even though they are important for the physiological and biological functions of the human body [8,9].

Fluorescence is the phenomenon demonstrated by various chemical constituents existing in the plant material. When exposed to ultraviolet

(UV) radiation or in the range of visible light, some constituents display fluorescence [10]. Identification of the powdered drug, extract or fractions of herbs can be carried out by utilizing the property of the organic molecules to absorb light over a specific range of wavelength and re-emit radiations [11]. Many phytochemical fluorescences are seen when suitably illuminated. The color of fluorescence is specific for each compound [12]. Some crude drugs are often assessed qualitatively because non-fluorescent substances may often be converted into fluorescent derivatives after reacting with different reagents and thus this is an important parameter of pharmacognostical evaluation [13].

Sphenoclea comprises two species but is the only genus in the plant family Sphenocleaceae (order Solanales). *Sphenoclea zeylanica* is an herb with spike of whitish flowers. *S. zeylanica* is native to the world tropics-wide but is also spread in moist areas of warm temperate and tropical zones including southern North America. It is differs from the family *Campanulaceae*, with which it is sometimes associated, by having overlapping corolla (petal) lobes in bud and a capsule that opens by a lid. *S. zeylanica* shoots are sometimes eaten with rice as it is a common weed of rice paddies. The objective of this study was to carry out the physicochemical parameters, fluorescence and heavy metals of methanol extract of leaves, stem, and flower of *S. zeylanica.*

Experimental

Plant collection authenticates by Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph's College, Trichy, Specimen No. SJCBOT4062.

METHODS

Physio chemical analysis

The physicochemical analysis includes number of parameters such as physical state, color, taste, the percentage of loss on drying as per standard method (0.5 ml). Crucible was placed on hot plate until fumes of sulfuric acid ceased to evolve. The crucible with sulfated ash was then heated in a muffle furnace at 600°C till the weight of the content became constant. Ash content as per method [14], ash value (water, alcohol, and acid soluble or insoluble ash) as per method [15,16].

Heavy metals

Na, Iron (Fe), Mg, Manganese (Mn), Lead (Pb), Zinc (Zn), Cadmium (Cd) and Copper (Cu) in plant samples were analyzed using atomic absorption spectrophotometer (AA 6300, Shimadzo, Japan) equipped with flame and graphite furnace. Air-acetylene flame was used for determination of metal content. The instrument was operated with the following conditions in flame mode: Acetylene 1.8 L/minutes, air 15 L/minutes, the inert argon gas flow and the temperature parameters were followed as recommended by the manufacturer. The absorption wavelength for the determination of each metal together with its linear working range and a correlation coefficient of calibration graphs are given in Table 2. Data were rounded off suitably according to the value of standard deviation from measurements in triplicate.

Fluorescence analysis

The powdered drug was examined under UV and ordinary light with different reagents. About 10 g of the powdered drug was taken in a petridish and treated with different reagents, namely, methanol, methanolic sodium hydroxide, 50% sulfuric acid, 50% nitric acid, 5% potassium hydroxide, 1 N hydrochloric acid, 1 N methanolic sodium hydroxide. These were observed under different wavelengths, i.e., visible rays and UV rays (254 nm and 365 nm). Various color radiations emitted were observed and noted [17].

RESULTS

The physicochemical parameters, fluorescence, and heavy metals were analyzed in the methanol extracts of leaf, stem and flower of *S. zeylanica.* The results obtained in each of this analysis were tabulated 1-5 and in Figs. 1-3.

DISCUSSION

Physico chemical study

The physicochemical parameters were studied in the methanol extracts of leaf, stem, and flower of *S. zeylanica.* To determine the quality and purity of a crude drug especially in a powdered form ash values are helpful. The objective of ashing vegetable drug is to remove all traces of organic matter that may otherwise interfere in an analytical determination. On incineration, a crude drug normally leaves an ash

Table 1: Physicochemical study of *Sphenoclea zeylanica* of leaf, stem and flower AQ1

S. No.	Parameter	M.L values obtained (%)	M.S values obtained (%)	M.F values obtained (%)
1	Total ash (%)	11.31	10.34	8.19
2	Water soluble ash (%)	14.6	11.05	10.27
3	Water insoluble ash (%)	10.3	8.21	5.16
4	Acid soluble ash (%)	7.40	6.27	5.09
5	Acid insoluble ash (%)	0.47	0.42	0.38
6	Sulphated ash (%)	17.3	16.76	15.82

M.L: Methanol leaf, M.S: Methanol stem, M.F: Methanol flower

Table 2: Heavy metal analysis of leaf, stem and flower in methanol extract of *Sphenoclea zeylanica*

S. No.	Elements analyzed	Amount of elements (in ppm)		
		Leaf	Stem	Flower
1	Fe	10.958	6.5873	0.2469
2	Cu	0.4263	0.3284	0.1210
3	Mn	0.8425	0.5294	0.3176
4	Zn	0.6486	0.5148	0.2314
5	Ni	0.0756	0.0541	0.0482
6	Co	0.0536	0.0247	0.0153
7	Pb	0.3486	0.3064	0.2046
8	Al	10.4961	9.1489	7.1965
9	V	0.4264	0.3759	0.4965
10	Cr	4.1059	3.4860	1.4067
11	Mo	0.7219	0.5489	0.4254
12	Hg	0.0075	0.0067	0.0051
13	As	0.0078	0.0046	0.0035
14	Cd	0.0581	0.0409	0.0394

Fe: Iron, Cu: Copper, Mn: Manganese, Zn: Zinc, Ni: Nickel, Co: Cobalt, Pb: Lead, Al: Aluminum, V: Vanadium, Cr: Chromium, Mo: Molybdenum, Hg: Mercury, As: Arsenic, Cd: Cadmium

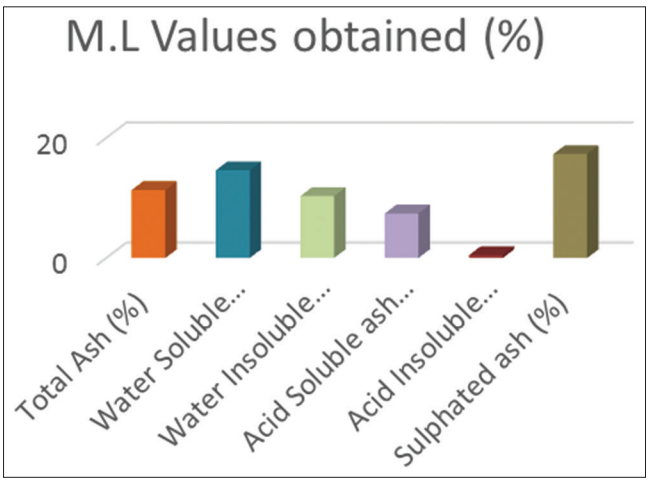

Fig. 1: Total ash percentage in leaf extract of *Sphenoclea zeylanica*

including carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium. The total ash of a crude drug reflects the care taken in its preparation. Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

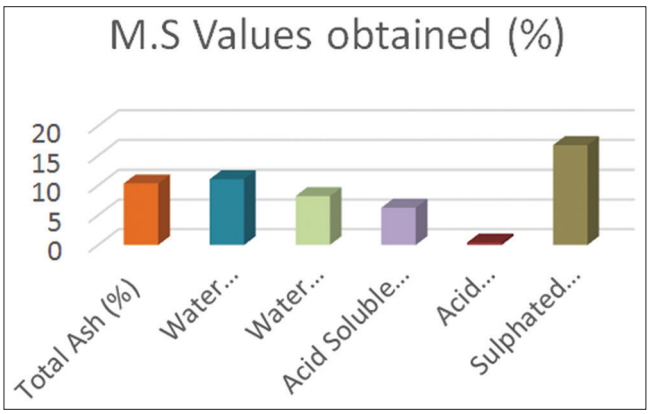

Fig. 2: Total ash percentage in stem extract of *Sphenoclea zeylanica*

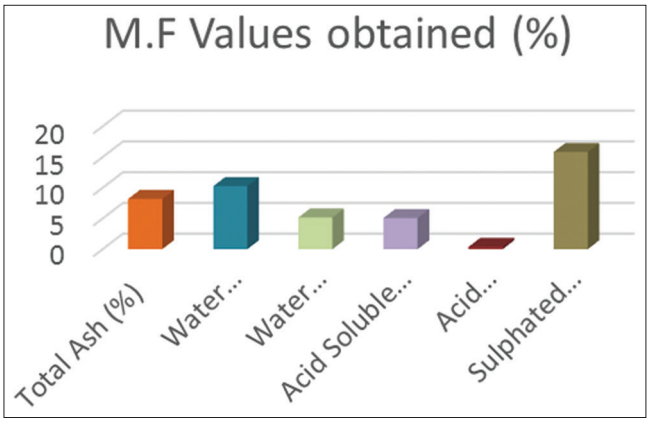

Fig. 3: Total ash percentage in flower extract of *Sphenoclea zeylanica*

The total ash percentage was found to be high in the leaf extract and was low in the flower extract of *S. zeylanica.* Water soluble ash and water insoluble ash were also higher in the leaf extract. The flower extract displayed only half the amount of water insoluble ash as that found in the leaf extract. Acid soluble and insoluble ash were also higher in the leaf extract of *S. zeylanica.* Very amount of acid-insoluble ash was seen in all the three extracts. The amount of sulfated ash in all three extracts of leaf, stem, and flower differed only by a close margin.

Heavy metal analysis

In plants, a number of mineral elements important to nutrition accumulate. Furthermore, other elements, which are not used directly by the plant such as Cd, Cobalt (Co), and Pb but are harmful to human health, also accumulate in these plants (Baker and Brooks, 1989; Lasisi *et al.,* 2005). A major concern is the environmental impact of these metals as well as their health effects. The availability of these metals in the soil affects their accumulation in plants (Khan *et al.,* 2007).

Cultivation, cross-contamination during processing or their deliberate introduction as therapeutic ingredients are responsible for heavy metal contamination of medicinal herbal products. One mechanism by which heavy metal contamination of herbal products has been documented is cultivation in soils containing high concentrations of heavy metals (Quig, 1998).

The heavy metal analysis was performed for the methanol extracts of leaf, stem, and flower of *S. zeylanica.* The elements analyzed were Fe, Cu, Mn, Zn, nickel (Ni), Co, Pb, aluminum (Al), vanadium (V), chromium (Cr), molybdenum (Mo), mercury (Hg), arsenic (As), and Cd. The amounts of these elements were recorded in ppm. It was noted that the elements Fe, Al and Cr occurred in the highest amount. Except for vanadium, all other metals were present in higher amount the leaf extract when compared to the stem and flower extract of *S. zeylanica.* In the flower extract, the amount of iron was <10 times the amount found in the leaf extract. Hg and arsenic, which are considered as toxic were found in very least amount.

Florescence analysis

The organic molecules absorbs light usually over a specific range of wavelength, get excited to a high energy level, and many of them emit such radiations while coming back to the original state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "fluorescence."

Fluorescence study report for leaves sample

The methanol extract of leaf of *S. zeylanica* were studied under different wavelengths and with different reagents to know their fluorescing

Table 3: Fluorescence study report for leaves sample AQ1

S. No.	Test	Observation under different wavelengths		
		White light	Long wavelength	Short wavelength
1	Sample+H_2SO_4	Dark green	Green	Light green
2	Sample+NaoH in water	Dark green	Red	Brick red
3	Sample+NaoH in methanol	Light straw	Light green	Straw
4	Sample+HCL	Green	Orange	Fluorescent orange
5	Sample+Water	Light straw	Light green	Straw

Table 4: Fluorescence study report for stem sample AQ1

S. No.	Test	Observation under different wavelengths		
		White light	Long wavelength	Short wavelength
1	Sample+H_2SO_4	Green	Brown	Dark green
2	Sample+NaoH in water	Red	Brick red	Green
3	Sample+NaoH in methanol	Straw	Green	Dark green
4	Sample+HCL	Green	Red	Brick red
5	Sample+Water	Light green	Green	Straw

Table 5: Fluorescence study report for flower sample AQ1

S. No.	Test	Observation under different wavelengths		
		White light	Long wavelength	Short wavelength
1	Sample+H_2SO_4	Pale	Green	Pale white
2	Sample+NaoH in water	Red	Brick red	Brown
3	Sample+NaoH in Methanol	Straw	Light straw	Brown
4	Sample+HCL	Green	Pale yellow	Dark green
5	Sample+Water	Light green	Green	Pale yellow

properties. With H_2SO_4, NaoH in water and HCL the leaf samples fluoresced green under white light. With H_2SO_4, the sample fluoresced green under long and short wavelengths also. With NaOH in water, the sample fluoresced red and brick red under long and short wavelengths respectively. However, with NaOH with methanol, the sample displayed straw colors when observed under white light and light of short wavelength. When viewed under long and short wavelengths, the sample fluoresced orange with HCl. With water, the sample displayed light straw, light green and straw colors under white light and lights of long and short wavelengths, respectively.

Fluorescence study report for stem sample

The stem sample of *S. zeylanica* was also studied to know its fluorescing properties with different reagents. With H_2SO_4, the sample fluoresced green with white and short wavelength lights. With NaOH in water, the sample fluoresced red and brick red under white and long wavelength lights. However with NaOH in methanol, the sample fluoresced straw, green and dark green for the three different wavelengths. Brick red color was observed for sample with HCl in short wavelength. With water, straw and green colors were observed.

Fluorescence study report for flower sample

The fluorescence properties were studied in the flower extract of *S. zeylanica.* It was seen that with H_2SO_4, the sample displayed pale colors under white and short wavelength light. When sample was combined with NaoH in water and Methanol, the brown color was observed when viewed under light of shorter wavelength. Pale yellow color was observed with HCl (under longer wavelength) and with water (under shorter wavelength).

CONCLUSION

Economically important plants that provide the basic raw materials for indigenous pharmaceuticals are represented by the medicinal plants. Plants present a great source of novel bioactive compounds with different activities, including anti-inflammatory, anti-cancer, antiviral, and antibacterial and cardioprotective activities. An essential step in establishing the standards is to identify the quality and purity of the drug which can be achieved through the pharmacognostical analysis. These characters help in evaluating the pharmacognostic value of the medicinal plants.

In our study, thephysicochemical parameters, fluorescence, and heavy metals of methanol extract of leaves, stem and flower of *S. zeylanica* were studied. The concentration of heavy metals differed for different parts of the plant *S. zeylanica*. Amounts of heavy metals detected were within permissible limits, and thus the plant can be used for the formulation of herbal remedies. The fluorescence study also showed significant results. Thus, the plant *S. zeylanica* can be used as a medicinal plant, and further research on the plant is recommended to know the bioactive compounds in the plant which can be harnessed to develop potent drugs.

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AQ1: Kindly cite tables 1, 3,4 and 5 in text part.