Pharmacognostical and preliminary phytochemical screening of the corm of *Stephania hernandifolia* (Willd.) Walp.

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ABSTRACT

The aim of the study is to cover the pharmacognostical and preliminary phytochemical screening of the corm of *Stephania hernandifolia* (Willd.) Walp. *Stephania hernandifolia* (Willd.) Walp. belonging to the family Menispermaceae is a natural taxon. The plant was collected from the Darjeeling district of West Bengal, India, during summer and was authenticated by the botanist. Pharmacognostical study included macroscopical characters, microscopical characters, physico-chemical constants and fluorescence analysis. Preliminary phytochemical screening includes phytochemical extraction, phytochemical testing and thin layer chromatography (TLC). In macroscopical studies of the corm, it was found that, it's shape was spherical, size was about 8 to 20 cm in length and 10 to 20 cm broad, colour was brown (Rusty), surface was rough, odour was not specific, taste was bitter, direction of growth was vertical, the surface has many circular and slightly raised spots. In microscopical studies of corm, it were found that it has periderm, vascular bundles, thin phelloderm cells, ground parenchymatous cells, dense masses of starch grains and wide circular secretory cavities were seen. In powder microscopy, sclereids and vessel elements have been observed with short sclerenchyma. Physico-chemical parameters like total ash value (13 % w/w), acid insoluble ash value (2.66 %w/w), water-soluble ash value (2.50 %w/w) and sulphated ash value (3.33 %w/w) were observed. Alcohol soluble extractive value (7.23 %w/w), water-soluble extractive value (10.84 %w/w) were also observed and loss on drying was observed as 2.50 %w/w. The foaming index was found to be 111.11. Preliminary phytochemical studies show the presence of alkaloids, carbohydrates, Steroids, Saponin, tannin and phenolic compounds flavanoids, and lignin in ethanolic extract and carbohydrates, Saponin, tannin and phenolic compounds flavanoids, and lignin in aqueous extract. Performing TLC of ethanolic extract using chloroform: ethanol (30:70) and methanol: water (70: 30) as solvent system, few spots were identified. The study helps in the correct identification of the plant. The presence of alkaloids and flavanoids explains that the plant must have valuable medicinal properties which must be explored.

Keywords: *Stephania hernandifolia* (Willd.) Walp, Macroscopical studies, Dense masses of starch grains and wide circular secretory cavities, Phytochemical screening.

INTRODUCTION

Modern phytochemical analysis of medicinal plants provides us an opportunity to explore all the medicinal plants in a rigorous manner. In India, it is believed that nature gives us suitable medicines for treatment of all types of diseases. Several hundred folk remedies are practiced in India even today. Most of the remedies are supposed to be cured by medicines, which may consist of a single plant or occasionally by several plants combined together, especially in complex cases where the treatment is done by considering the root cause of each 'trouble'. About 18,000 species of flowering plants have been reported within the geographical boundaries of India [1]. According to the 'World Bank' the number of medicinal plants available in India is approximately 2500. Many of these plants are traditionally used in India for the treatment of diabetes mellitus. Proper investigation of such plants may provide invaluable antidiabetic drugs in standardized forms. For diabetes, herbal medicine is preferred to synthetic one, primarily for the following reasons: (1)

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herbal drugs are less toxic and have fewer side effects, (2) insulin cannot be used orally and continuous insulin injection may create many side effects and toxicity, and (3) oral synthetic drugs have several limitations. The purpose of the present project work is to make a comprehensive study of a medicinal plant named *Stephania hernandifolia* (Willd.) Walp., used for diabetes treatment in India [2].

MATERIAL AND METHODS

Collection of the specimen:

The plant species for the proposed study has been collected from Darjeeling district of West Bengal. Proper care was taken to select healthy plants with normal organs. Necessary samples of different organs were cut and removed from the plant and were fixed in FAA (farmalin- 5ml + acetic acid -5ml + 70% ethyl alcohol 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary- butyl alcohol as per the schedule given by Sass (1940) [3]. Infiltrations of the specimens were carried out by gradual addition of paraffin wax (melting point 58-60^{0}C) until tertiary butyl alcohol (TBA) solution attained supersaruration. The specimens were then cast into paraffin blocks.

Fig 1: Shoot and Root of *Stephania hernandifolia* (willd.) Walp.

Sectioning:

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thicknesses of the sections were 10-12 µm. Dewaxing of the sections was done by customary procedure given by Johanson, (1940) [4]. The sections were stained with toluidine blue as per the method published by O' Brien *et al* (1964) [5]. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also noticed. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies etc. Wherever necessary, sections were also stained with safranin.

Photomicrographs:

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Therefore, photographs of different magnifications of the corm were taken with Nikon Labphot 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appeared bright against dark background. Magnifications of the figures have been indicated by the scale-bars. Descriptive terms of the anatomical features are taken from the standard Anatomy books [6-10].

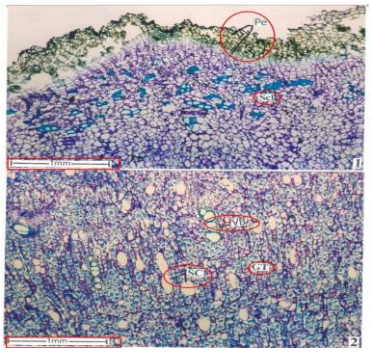

Figure 2: Anatomy of the corm of *Stephania hernandifolia* (willd.) Walp.

1. T.S of corm Periderm and Sclereids, 2. T.S of corm showing outer Vascular bundles; GT- Ground tissue, Pe- Periderm, SC- Secretory cavity, Scl- Sclerenchyma, VB- Vascular bundle

The residue left after incineration of a drug is designated as ash. The residue originate from inorganic elements present in the plant is called as physiological ash. It varies with in definite limits according to types of soil, dust, sand and mineral impurities and

admixture of other drugs may alter the ratio. Ash value represents the inorganic salts naturally occurring in the drug and adhering to it. Total ash is the residue remaining after incineration. The acid insoluble ash is the part of total ash which is insoluble in dilute hydrochloric acid. Mixing of sulphuric acid with powdered crude drug before ashing and this sulphated ash is normally less fusible than ordinary ash. The moisture content was determined in reference to air-dried sample by loss on drying method.

Extractive value which is an indicative of approximate measures of chemical constituents and nature of the constituents was performed using ethanol and distilled water as solvents [11-12].

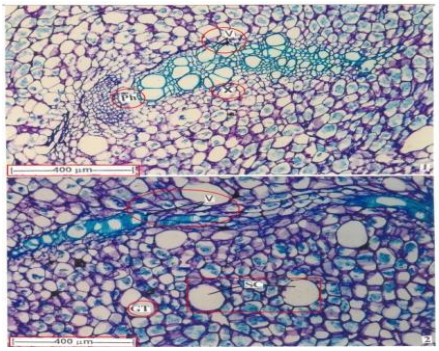

Figure 3: Structures of vascular bundles of corm of *Stephania hernandifolia* (willd.) Walp.

1. Inner Vascular bundles enlarge, 2. Vessel element in L.S. view; GT- Ground tissue, Ph- Phloem, SC- Secretory cavity, V- Vessel, X- Xylem

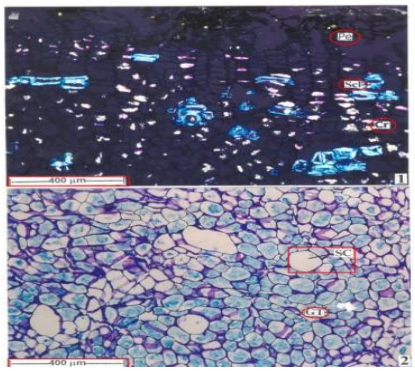

Figure 4: Structures of sclereids and ground tissue of corm of *Stephania hernandifolia* (willd.) Walp.

1. Crystals and sclereids under Polarized light microscope,
2. Ground tissue and Secretory cavities

Cr- Crystals, GT- Ground tissue, Pe- Periderm, SC- Secretory cavity, Scl- Sclerenchyama

Successive extraction was done in soxhlet extractor using the following solvents: ethanol and distilled water. Both ethanolic and aqueous extracts obtained by extraction of the powdered corm of *Stephania hernandifolia* (Willd.) Walp., and were subjected to various qualitative tests for the identification of various plant constituents present in the species. Details of these tests performed to make a qualitative phytochemical analysis, are given in the table [11].

Ascending thin layer chromatography was performed for the separation of phytocontituents. The extracts which showed presence no of phytoconstituents present in each extract. The mobile phases used were, chloroform : ethanol (30:70) and methanol : water (70: 30). Different spots developed in each solvent system were identified through UV light (λ max 254 nm) and the Rf values were accordingly calculated [13-14].

S. No.	Parameters	% w/w
1.	Ash values	
	(i) Total Ash	13
	(ii) Acid Insoluble Ash	2.66
	(iii) Water Soluble Ash	2.50
	(iv) Sulphated Ash	3.33
2.	Extractive Values	
	(i) Alcohol Soluble Extractive	7.23
	(ii) Water soluble Extractive	10.84
3.	Loss on Drying	2.50

Table 1: Physico-chemical parameters of powdered of corm of *Stephania hernandifolia* (willd.) Walp.

RESULTS AND DISCUSSION

In macroscopical studies of the corm, it were found that it's shape was spherical, size was about 8 to 20 cm in length and 10 to 20 cm broad, colour was brown (Rusty), surface was rough, odour was not specific, taste was bitter, direction of growth was vertical, the surface has many circular and slightly raised spots. In microscopical studies of corm, it were found that it has periderm, vascular bundles, thin phelloderm cells,

ground parenchymatous cells, dense masses of starch grains and wide circular secretory cavities were seen. In powder microscopy, sclereids and vessel elements have been observed with short sclerenchyma.

Plant constituents	Extracts	
	Ethanolic extract	Aqueous extract
Alkaloids	+	-
Carbohydrates	+	+
Glycosides	-	-
Steroids	+	-
Saponins	+	+
Tannins and Phenolic compounds	+	+
Proteins and Amino acids	-	-
Gums and Mucilage	-	-
Flavonoids	+	+
Triterpenoides	-	-
Fixed Oils and Fats	-	-
Lignin	+	+

Table 2: Qualitative phytochemical analysis of various extracts of powdered corm of *stephania hernandifolia* (willd.) walp

Physico-chemical parameters like total ash value (13 % w/w), acid insoluble ash value (2.66 %w/w), water-soluble ash value (2.50 %w/w) and sulphated ash value (3.33 %w/w) were observed. Alcohol soluble extractive value (7.23 %w/w), water-soluble extractive value (10.84 %w/w) were also observed and loss on drying was observed as 2.50 %w/w. The foaming index was found to be 111.11

Qualitative phytochemical analysis and Thin Layer Chromatography were investigated. The shade dried corm of *Stephania hernandifolia* (Willd.) Walp., were extracted with ethanol and distilled water by continuous hot soxhlet extraction and the percentage yield were found to be 10.01 % w/w (ethanol extract) and 16.67 % w/w (aqueous extract).

The extracts obtained were subjected to qualitative phytochemical tests to find out the active constituents, which showed presence of alkaloids, carbohydrates, tannins and phenolic compounds, steroids, flavonoids, saponins and lignin in ethanolic extract and carbohydrates, tannins and phenolic compounds, flavonoids, saponins and lignin in aqueous extract of powdered corm of *Stephania hernandifolia* (Willd.) Walp.

All the above extracts were subjected to Thin Layer Chromatography and further identified the presence of the phyto constituents. Ethanolic extract showed three spots with Rf values 0.40, 0.55 and 0.75 and aqueous extract showed three spots with Rf values 0.65, 0.75 and 0.90 respectively.

Sl. No	Extracts	Solvent systems	No of spots	Color of spots	Rf Values
1.	Ethanolic extract	chloroform : ethanol (30 : 70)	3	Light green, Green, Deep green	0.40 0.55 0.75
2.	Aqueous extract	methanol : water (70 : 30)	3	Light blue, Blue, Deep blue	0.65 0.75 0.90

Table 3: TLC of various extracts of powdered corm of *Stephania hernandifolia* (willd.) Walp

CONCLUSIONS

The pharmacognostical study which includes macroscopy, microscopy and physic chemical constants gives valuable information. This will help for correct identification of this plant for future investigation. The preliminary phytochemical studies show the presence of alkaloids, carbohydrates, tannins and phenolic compounds, steroids, flavonoids,

saponins and lignin, which may responsible for Antidiabetic activity and others activities. Further investigation on the isolation and identification of phytocomponent(s) in the plant may lead to chemical entities with potential for clinical use.

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