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Pharmacognostic Studies of *Alternanthera philoxeroides* (Mart.) Griseb

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Abstract
Alternanthera philoxeroides, commonly known as alligator weed, is an emergent aquatic plant belongs to the family Amaranthaceae. *Alternanthera philoxeroides* is an ornamental plant found throughout India. The methanolic extract of whole plant was evaluated for antinociceptive and antihyperglycemic activities. In present investigation the detailed Pharmacognostic study of *Alternanthera philoxeroides* is carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening and physicochemical evaluation.

Keywords: *Alternanthera philoxeroides*, pharmacognosy, microscopy

Introduction
Medicinal plants are an important source for the therapeutic remedies of various diseases. In India, from ancient times, different parts of medicinal plants have been used to cure specific diseases. India is known for its rich diversity of medicinal plants and hence called, Botanical Garden of the World. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. Recently, natural phytochemicals have gained a lot of attention as they have shown tremendous advantages in human health.

Green Leafy Vegetables also contain an immense variety of bioactive non-nutritive health enhancing factors such as antioxidants, phytochemicals, essential fatty acids and dietary fiber [2]. Due to their dietary importance, many scientific studies have been carried out on the nutritive values of green leaves [3]. However a key obstacle, in acceptance of alternative medicine in developed countries, is the lack of proper standardization, documentation and stringent quality control. Hence there is a need for documentation of research work carried out on traditional medicines.

Alternanthera philoxeroides (Mart.) Griseb. (Amaranthaceae) is a perennial herb, aquatic plant which originated in South Africa and now seen in many parts of the world. The plant is reported for only few pharmacological activities. The plant was reported for preventive and therapeutic effects against influenza [4]. The methanol extract of whole plant was reported for antinociceptive and antihyperglycemic activities in mice [5]. Aqueous extract of the plant demonstrated for inhibitory activity against human immunodeficiency virus [6]. The plant extracts has been found to possess inhibitory activity against dengue virus *in-vitro* [7].

The reported phytoconstituents of the plant include phaeophytin a, phaeophytin a', oleanolic acid, β-sitosterol, 3β-hydroxystigmast-5-en-7-one, α-spinasterol, 24-methylene cycloartanol, cycloeucalenol and phytol [8]. The antitumour compounds alternanthin B and N-trans-feruloyl-3,5-dimethoxytyramine has been isolated from aerial parts of *A.philoxeroides* [9]. Since the standardization of the plant have not yet been reported the present study has been designed to establish pharmacognostical study of leaves of *A.philoxeroides* include its morphological, anatomical and biochemical characteristics.

Materials and Methods
Plant Material
The fresh leaves of *Alternanthera philoxeroides* were collected from local market of Guntur, Andhra Pradesh, India. The plant specimen was sent to Botanical Survey of India, Ministry of Environment & Forests, Government of India, Southern Regional Centre, Coimbatore for authentication. The plant specimen was identified as *Alternanthera philoxeroides* (Mart) Griseb. The healthy leaves were shade dried and powdered to a get coarse powder.

Pharmacognostical evaluation includes macroscopical and microscopical characters. Quantitative leaf microscopy to determine stomatal number, stomatal index and physico-chemical parameters of the powdered drug such as ash value, extractive value and loss on drying were performed. Examination of powder starch grains, calcium oxalate crystals were carried out as per standard procedure [10-12]. Chemical tests were employed in preliminary phytochemical screening for various secondary metabolites such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, sterols, tannins.

Physicochemical Characters
Physico-chemical parameters of the powdered drug such as ash value, extractive value and loss on drying were performed. Physicochemical studies revealed moisture content, foreign matter, total ash, acid insoluble ash, water soluble ash, sulphated ash.

Determination of Moisture content (loss on drying)
Place about 10g of drug (without preliminary drying) after accurately weighing (accurately Weighed within 0.01g) it in a tarred evaporating dish. For example, for underground or unpowdered drug, prepare about 10g of the sample by cutting shredding so that the parts are about 3mm in thickness. Seeds and fruits, smaller than 3mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tarred evaporating dish dry at 105 ^{0}C for 5 hours, and weigh.
Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.01 g difference.

Determination of Foreign matter
Weigh 100 – 500 g of the drug sample to be examined or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of lens (6xs). Separate and weigh it and calculate the percentage present

$$\% \text{ of foreign matter} = \frac{\text{Amount of foreign matter} \times 100}{\text{Amount of drug taken}}$$

Ash Value Determination
Determination of Total Ash
Incinerate about 2 to 3 gm accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450 ^{0}C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450 ^{0}C. Calculate the percentage of ash with reference to the air dried drug.

Determination of Acid Insoluble Ash
Boil the ash obtained in total ash for 5 minutes with 25 ml of *dilute hydrochloric acid*, collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Determination of Water Soluble Ash
Boil the ash for 5 minutes with 25ml of water, collect insoluble matter in a Gooch crucible, or an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450 ^{0}C. Subtract the weight of the insoluble matter from the weight of the ash obtained. Calculate the percentage of water – soluble ash with reference the air dried drug.

Extraction of phytoconstituents
The powdered leaf material was extracted with solvents like methanol by cold maceration process. The extract were prepared by taking 50g of dried leaves powder in separate containers and to this 200mL of methanol was added and kept in a shaker for 24 h. The extract was collected by filtered through 5 layers of muslin cloth. The extraction process was repeated twice. Then the collected filtrates were pooled, concentrated and dried at mild temperature.

Preliminary phytochemical screening
The phytochemical screening for the extracts was carried out by standard protocols [13]. Alkaloids (Mayer's test), glycosides (Legal's test), saponins (froth formation test), carbohydrates (Molisch's test), proteins (Xanthoproteic test), aminoacids (Ninhydrin test), Flavonoids (Lead acetate test), steroids (Salkowski test), tannins (Ferric chloride test) were analyzed.

Result
Morphological Characters
Alternanthera philoxeroides is a Perennial herb; stems creeping or floating ascending towards apex, rooting at the lower nodes, branched, hollow, with a longitudinal hairy groove on two opposite sides. Leaves and stems vary greatly in size and shape. Fleshy, succulent stems can grow horizontally and float on the surface of the water, forming rafts, or form matted clumps which grow onto banks. The leaves are opposite in pairs, with a distinctive midrib, and range in size from 5-10cm.
Macroscopically, the *Alternanthera philoxeroides* is compound leaf, lanceolate shape, acute apex, entire margin, glabrous surface, graduate base, short solid petiole. The macroscopical characters were shown in the table: 1.

Table-1: Morphology of *Alternanthera philoxeroides* leaves

Morphological Parameters		Observation
Size:	Length:	9.4cms
	Width:	1.4cms
	Shape	Lanceolate
	Apex	Acute
	Margin	Entire
	Base	Aduate
	Petiole	Short
	Surface	Glabrous
Colour:	Inner:	Dark green
	Outer:	Light green
	Odour	Characteristic

Fig 1: *Alternanthera philoxeroides*

Microscopical Characters
Microscopically Transverse section of leaf consists of lamina and midrib region. Lamina exhibits upper and lower epidermis. Epidermis covered with cuticle. Trichomes are present on both epidermises. Diacytic stomata found on epidermis, mesophyll comprises of palisade and spongy parenchyma. Palisade cells are columnar one layered. Midrib exhibits arc shaped vascular bundle enclosed by pericyclic fibre. Vascular bundle consists of xylem and phloem. The transverse section is shown in the figure: 2.

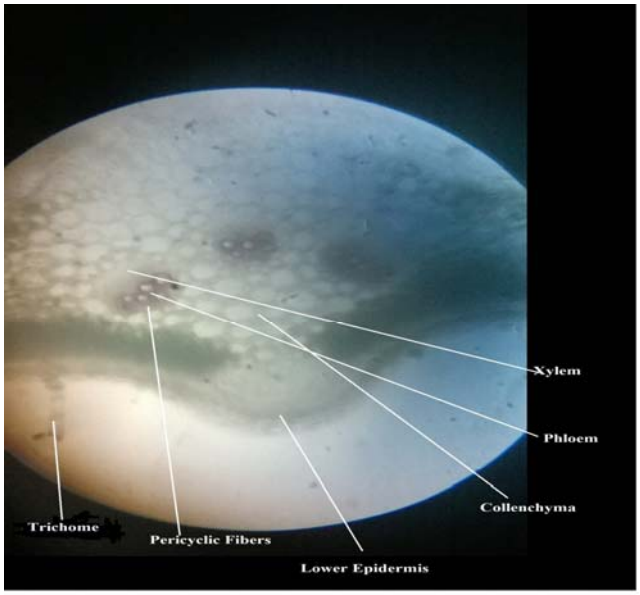

Fig-2: Transverse section of leaf of Alternanthera philoxeroides

Quantitative leaf microscopy

Quantitative leaf microscopy is used to determine the stomatal number, stomatal index. Fragment of lamina showing stomata and venation. Stomatal index was found for upper and lower epidermis is 33%. Stomatal number for upper epidermis is 15 and lower epidermis is 18 (Figure: 3).

Fig-3: Diacytic Stomata

Physicochemical characters

Physicochemical studies revealed moisture content, foreign matter, total ash, acid insoluble ash, water soluble ash, sulphated ash (table-2). These are helpful in detecting the adulterants.

Table 2: Physicochemical constants of leaves of *Alternanthera philoxeroides*

Parameter	Physico-chemical Constant (%w/w)
Moisture content	10%
Foreign matter	0.007%
Total ash	87%
Acid insoluble ash	5%
Water soluble ash	2%
Sulphated ash	1.8%

Preliminary phytochemical screening:

The results of preliminary phytochemical screening revealed the presence of various phytoconstituents (table-3). The presence of carbohydrates, aminoacids, proteins, cardiac glycosides, steroids, alkaloids, flavonoids, total phenolics and tannin contents were reported. The preliminary phytochemical screening will be useful to know the genuinity of the drug.

Table -3: Preliminary Phytochemical Screening of *Alternanthera philoxeroides leaves*

Name of the Test	Methanolic Extract
Carbohydrates	+
Proteins	+
Aminoacids	+
Steroids	+
Cardiac glycosides	+
Flavonoids	+
Alkaloids	+
Tannins & phenolic compounds	+

Conclusion

The present study on pharmacognostical characters of leaves of *Alternanthera philoxeroides* (Mart.) Griseb may be useful to supplement information with regard to its identification and will be helpful in establishing standardization criteria. These simple reliable standards will be useful to use herbs as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production.

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