



Pharmacognostical Studies of *Amaranthus Spinosus* Linn

Vipin Chandra Pal*, Om Veer Singh, Bhuwanendra Singh, Arshad Ahmad

Radha Govind Institute of Pharmacy, Chandausi, Moradabad (UP), India

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*Corresponding Author:

E-mail: vipinpalrt@gmail.com

Tel.: +919634555671

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Abstract

Amaranthus spinosus leaves species has been widely used as medicine in Ayurvedic system of medicine. Its leaves are used in treatment of malaria, hepatic disorders, fever, inflammation, leprosy, eczema, leucorrhoea and bronchitis. In view of its medicinal importance and taxonomic confusion, so here we determined the, microscopical structures along with powder characters of malaria, hepatic disorders, fever, inflammation, leprosy, eczema, leucorrhoea and bronchitis leaves. Fluorescence analysis, physico-chemical constants (total ash, acid insoluble ash, water soluble ash, ether soluble extractive, water soluble extractive, alcohol soluble extractive, foaming index) of leaves powder. Preliminary phytochemical investigation was carried out for the various crude extracts using solvent of different polarity. These studies provided referential information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario of lack of regulatory laws to control quality of herbal drugs.

1 Introduction

Amaranthus spinosus (Family Amaranthaceae) known in Hindi as Cauleyi or Kateli, is an erect, spinous, annual or perennial herb found throughout India. The plant is a constituent of an herbal drug LEUCOSOL-H which is found to be effective in Leucorrhoea. The grain was found to be a nutritious pseudo cereal, yielding high energy, starch and protein. It can be utilized in the development of acceptable value added products with high nutritive value. The leaves contain high amount of oxalic acid, 1161.4 mg/100 g. In ancient India, a paste of the roots prepared in rice-wash was used by women for sterilization after menstruation. The seed oil contains a relatively high concentration (2.4-8.0%) of squalene. The oil also contains relatively high concentration of tocotrienols, a rare form of Vitamin E, which is reported to inhibit 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the key regulatory enzyme in cholesterol biosynthesis. In folk medicine, this plant is used for the treatment of a variety of diseases such as malaria, hepatic disorders, fever, inflammation, leprosy, eczema, leucorrhoea and bronchitis.

Microscopy is an important tool in the evaluation of crude drugs which is applicable at various leaves such as the authentication of the crude drugs, study of powdered drugs visualizing calcium oxalate crystals.

Microscopy method allows more detailed examination of a drug. It can be used to identify the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered form. Ash values, extractive values and foaming index are used for the study of physical properties¹⁻³.

No report is available on micro-morphological work of this drug. Hence, it is felt desirable to pursue a study on pharmacognostical investigation of *Amaranthus spinosus* leaf to supplement useful data in regard to its correct identity of this plant.

2 Materials and Methods

2.1 Plant material

The leaves of *Amaranthus spinosus* was collected from Dharmapuri, Tamil Nadu in the month of June. The collected material was identified and authenticated by Dr. P. Jayaraman, botanist, Plant Anatomy Research Centre (PARC), Chennai. The fresh leaves were separated and used for the study of microscopical characters; whereas dried leaves powder material was used for the determination of physico-chemical parameters and phytochemical constituents.

Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in 5 ml of formalin, 5 ml of acetic acid, and 90 ml of 70% v/v ethyl alcohol (FAA). After 24 hours of fixing the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the method⁴. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

2.2 Sectioning

The paraffin embedded specimens were section with the help of rotary microtome. The thickness of the sections was 10-12 micrometers. Dewaxing of the sections was by customary procedure⁵. The sections were stained with toluidine blue as per the method published⁶. Since toluidine blue is a polychromatic stain, the staining results were remarkably good, and some cytochemical reactions were also obtained. The rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies. Wherever necessary sections were also stained with safranin and fast green and Iodine (for starch).

2.3 Photomicrograph

Microscopic description of tissues or supplemented with micrographs were necessary photographs of different magnifications 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 appear bright against dark backgrounds. Magnifications of the figures are indicated by the scale bars⁷⁻⁹.

2.4 Physico-chemical parameters

Physico-chemical parameters of *Amaranthus spinosus*, leaves powder were determined and reported as total ash, water soluble ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive and moisture content¹⁰.

Fluorescence analysis was carried out for the leaves powder, treated with different chemical reagents in porcelain dish. Leaves powder treated with chemical reagents were placed under visible and UV light (254 nm and 366 nm) for fluorescence emitted by the sample^{11,12}.

2.5 Preparation of extracts

Dried powder of leaves was exhaustively extracted successively in soxhlet apparatus, using hexane, ethanol and distilled water respectively¹³. The extracts were then made to powder by using rotary evaporator under reduced pressure¹⁴.

2.6 Preliminary phytochemical studies

Preliminary phytochemical test of various extracts of *Amaranthus spinosus* bark were performed and the chemical constituents were detected¹⁵.

3 Results and Discussions

3.1 Microscopy study

3.1.1 Anatomy of the leaf

The leaf has strongly protrude abaxial midrib with deep adaxial concavity and thin lamina. The midrib was 395 mm in vertical axis and 500 mm in horizontal axis. The epidermal layer of the midrib was thin with small squish cells and thick cuticle. The ground tissue consists of four or five layers polyhedral, thin walled, compact parenchyma cells (Fig1.1 & 1.2).

The vascular bundle was fairly large and in shaped. It was collateral with adaxial xylem and abaxial phloem (Fig1.2, 2.1). The xylem elements are in clusters with wide, thick walled, angular metaxylem elements and one or two protoxylem elements (Fig 2.1). The Metaxylem elements are 20 mm wide, angular, sieve elements and scatters, solitary fibers.

3.1.2 Lamina

The lamina was iso-bilateral having palisade tissue both on the abaxial and adaxial sides (Fig 2.2). The palisade cells were two or three layer on the adaxial side and two layers with short cells on the abaxial side. Large carities were present, the palisade tissue in which druses were present. In between the palisade layers and along the median zone occurs large spherical darkly staining mesophyll tissue. The spongy mesophyll tissue surrounding the vascular bundle was called "Kranz tissue" (Fig 4.2.3).

3.1.3 Cell inclusion

Calcium oxalate crystals were abundant in the midrib (Fig 3.1) and also in mesophyll tissue (Fig 3.2.3). Crystals are druses or spherocrystals, and druses of different sizes occur in various tissue. In ground parenchyma cells the druses were spherical and large, measuring 50 mm in size and also measuring 10 mm in diameter.

Apart from the druses minute particles of crystals also occur within the xylem elements (Fig 3.1.2).

3.1.4 Stomata

Stomata are in variably "anomocytic" type no distinct subsidiary cells were seen surrounding the stoma. The guard cells were elliptical with wide stomatal pore (Fig: 4.1). The abaxial epidermal cells were thin walled with wavy "zig-zag" cells walls (Fig 4.1)

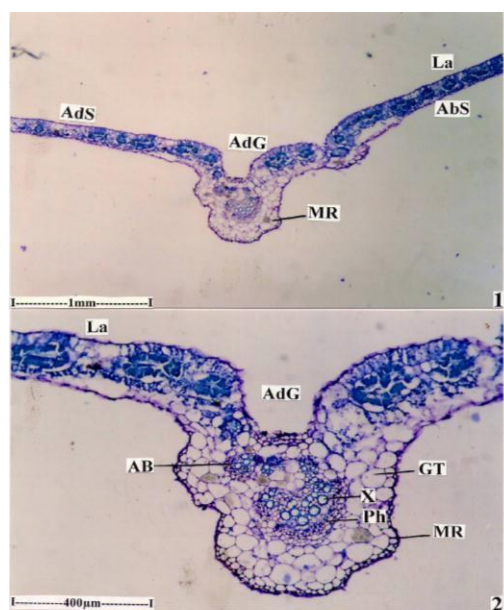


Fig 1: Anatomy of the leaf

1.1: T.S. of leaf through midrib with lamina, 1.2: T.S. of midrib with lamina (AdG – Adaxial groove, Abs – Abaxial side, AdS – Adaxial side, AB – Accessory bundle, GT – Ground tissue, La – Lamina, MR – Midrib, Ph – Phloem, X – xylem)

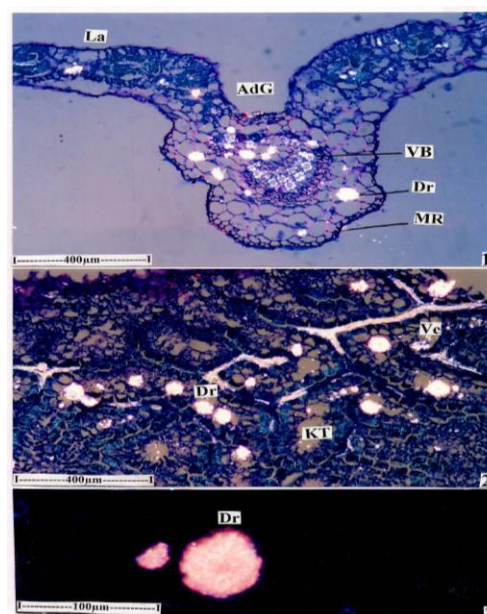


Fig 3: Crystal distribution in the leaf (Under polarized light microscope)

3.1: T.S. of midrib showing crystals in the ground tissue, 3.2: Paradermal section showing crystal distribution in the mesophyll tissue, 3.3: A Druses in the mesophyll tissue magnified (AdG – Adaxial groove, Dr – Druses, KT – Kranz tissue, La – Lamina, MR – Midrib, VB – Vascular bundle, Ve – Vein)

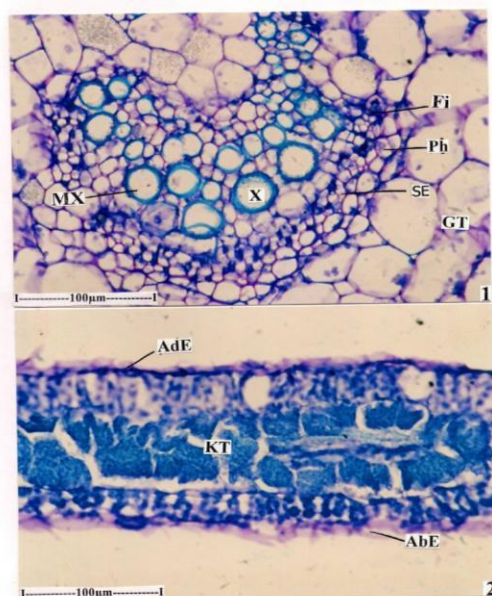


Fig 2: Structure of the midrib and lamina

2.1: T.S. of midrib – Vascular bundle enlarged, 2.2: T.S. of lamina (AbE – Abaxial epidermis, AdE – Adaxial epidermis, Fi – Fibres, GT – Ground tissue, KT – Kranz tissue, MX – Metaxylem, Ph – Phloem, SE – Sieve element)

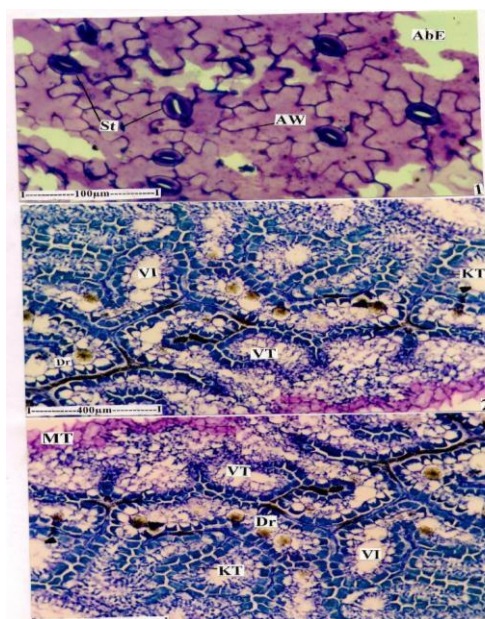


Fig 4: Paradermal section of the leaf showing stomato Morphology and venation pattern

4.1. Abaxial epidermis with stomata, 4.2&3 Vein – islets and Vein – termination (AbE – Abaxial epidermis, AW – Anticlinal wall, Dr – Druses, KT – Kranz tissue, MT – Mesophyll tissue, VI – Vein – islets, VT – Vein – termination, St – Stomata)

3.1.5 Venation

Venation pattern was studied both in paradermal section and leaf powder. In paradermal of dark “Kranz-tissue” was observed. The vein-islets are fairly distinct: large and irregular in outline. The vein-terminations are distinct, forked once or twice (Fig 4.2.3).

3.1.6 Powder microscopy

The leaf powder mostly epidermal peelings were found in small fragments. They were stained with safranin for microscopic studies.

Adaxial epidermis

The adaxial epidermal cells were small. The cells were thick walled, polygonal in outline with straight anticlinal walls. Stomata were also seen on the adaxial epidermis. They were circular in outline and stomatal were not evident (Fig 5.1)

Abaxial epidermis

Fragment of abaxial epidermis has different structure from the adaxial epidermis the stomata were more in number on the abaxial epidermis. They were elliptical in shape (Fig 5.2.3) the cell walls were also wavy and the cells were “ameboide” in outline.

Venation pattern

Fragments of lamina stained with “Toluidine blue” were studied. The vein-islets are rectangular somewhat parallel to each other having thick veins. The vein-terminations were distinct, long, slender and forked (Fig 6.1.2).

Druses

Large spherical, darkly stained masses of druses are diffuse were also seen within the vein-islets. The druses were diffuse in distribution and mostly solitary (Fig 6.1.2).

Trichome

Muticellular, uniseriate, unbranched, epidermal tichomes were frequently seen on the lamina. The cells of the trichomes were vertically oblong and wide of the trichome was dilated into hemispherical body (Fig 6.3).

3.2 Physico-chemical parameters

The powder of the leaves was preliminary evaluated by determining physical constants like total ash, water soluble ash, acid insoluble ash, ether, alcohol and water soluble extractive, moisture content and foaming index (Table 1).

The powder showed mostly green colour in daylight. Green fluorescence was mostly observed in U.V. light at 254 nm (Table 2).

Physico-chemical parameters and qualitative chemical tests will be useful tool along with microscopical characteristics of *Amaranthus spinosus* leaves.

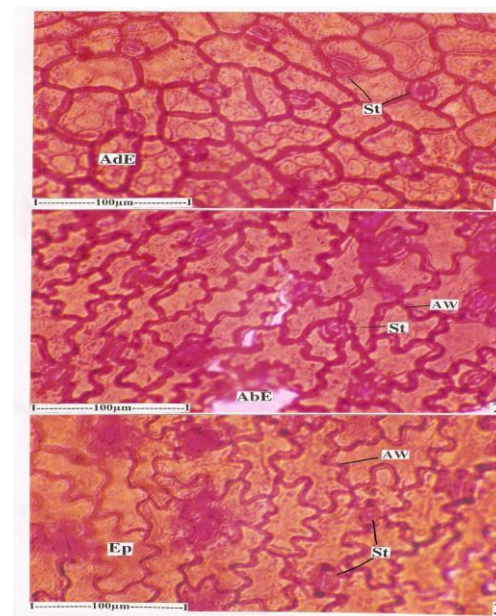


Fig 5: Powder Microscopy of the leaf

5.1: Adaxial epidermis with stomata, 5.2&3 Fragments of abaxial epidesmis with stomata
(AbE – Abaxial epidermis, AdE – Adaxial epidermis, Aw – Anticlinal Wall)

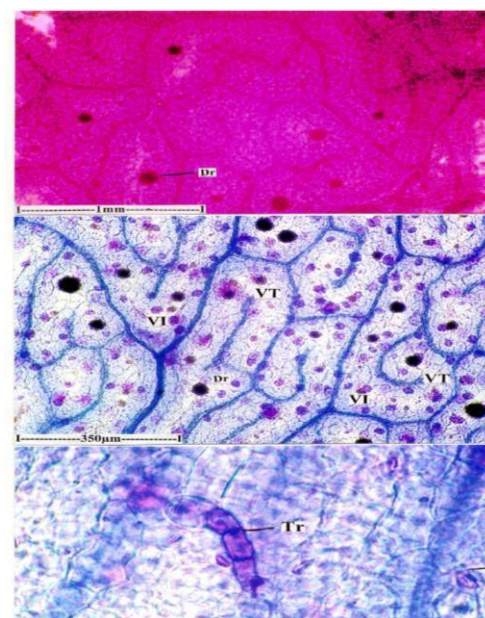


Fig 6: Venation pattern

6.1: Fragment of lamina showing druses distribution (stained with safranin), 6.2: Venation pattern with druses distribution (stained with toluidine blue), 6.3: A covering trichome on the epidermal cell
(Dr – Druses, St – Stomata, VI – Vein – islets, VT – Vein – termination)

3.3 Preliminary phytochemical studies

The qualitative chemical test of *Amaranthus spinosus* leaves extract showed the presence of alkaloids, glycosides, steroids, triterpenoids, tannins, flavonoids, gums and mucilage. The results are reported in (Table 3).

Table 1: Physico-chemical parameters of *Amaranthus spinosus* leaves powder

Physicochemical Parameters		Values
Ash Values	Total Ash	9.25 % w/w
	Water Soluble Ash	2.21 % w/w
	Acid Insoluble Ash	0.92 % w/w
Extractive values	Ether soluble extracts	1.78 % w/w
	Alcohol soluble extracts	11.50% w/w
	Water soluble extracts	27.36 % w/w
Moisture Content		9.64 % w/w
Foaming Index		142.85

Table 2: Fluorescence analysis of *Amaranthus spinosus* leaves powder

Treatment	Day light	U.V. Light (254nm)	U.V. Light (366nm)
Powder as such	Green	Green	Green
Powder + 1 N HCl	Colorless	Colorless	Colorless
Powder + aqueous 1 N NaOH	Yellowish Green	Fluorescence Yellowish Green	Yellowish Green
Powder + alcoholic 1 N NaOH	Green	Fluorescence Green	Fluorescence Green
Powder + 50% HNO ₃	Light Yellow	Fluorescence Green	Fluorescence Green
Powder + 50% H ₂ SO ₄	Pale Brown	Greenish Brown	Brown
Powder + Methanol	Dark Green	Green	Green
Powder + Water	Off Colour	Green	Green

Table 3: Preliminary phytochemicals screening of different extracts of *Amaranthus spinosus* leaves

Extract	Hexane	Ethanol	Aqueous
Steroid	+	-	-
Triterpenoid	+	-	-
Flavonoid	-	+	+
Quinonoid	-	-	-
Furaminoid	-	-	-
Tannin	-	+	-
Glycosides /Sug	-	+	+
Alkaloid	-	+	-
Coumarin	-	-	-
Gum and mucilage	-	-	+

(+)-Presence of phytochemical compounds; (-)-Absence of phytochemical compounds

4 Conclusions

This study on micro morphological features of *Amaranthus spinosus*, proposed a set of anatomical parameters may enables those who handle this plant to maintain its quality control.

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