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Pharmacognostical characteristics of *Amaranthus viridis* L

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Abstract

Establishment of Pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification. Evaluation of the fresh, powdered and anatomical sections of the *Tecomastans* leaves were carried out to determine themicromorphological, numerical and phytochemical profiles. The leaf of *Amaranthus viridis* L is compound leaf, alternate, ovate, entire, glabrous, decurrent base. In the microscopic studies, the leaves showed the presence of trichomes, collenchyma, vascular bundles, spongy parenchyma, palisade cells and stomata. Phytochemical evaluation revealed the presence of tannins, phenols, cardiac glycosides, carbohydrates, alkaloids, Saponin, protein and amino acids, flavonoids, steroids. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.

Keywords: *Amaranthus viridis* L pharmacognostic profile, standardization, phytochemical investigation

Introduction

Amaranthus viridis Linn (Amaranthaceae) is an annual herb with an upright, light green stem that grows to about 60-80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3-6 cm long and 2-4 cm wide, long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers. It requires well drained fertile soil in a sunny position. It should not be provided with inorganic fertilizer. It is cultivated as a food in tropical countries. It photosynthesis by C₄ carbon-fixation pathway which effects at high temperature. In India it is known as Kuppaikeerai in Tamil language, kuppacheera in Malayalam language, Jungalichaulayl in Hindi, Chilaka-thotakoora in Telugu. The herb is used as astringent, emollient, in dysentery, inflammation, constipation, eczema, bronchitis, antidiabetic, anaemia and leprosy and Leaves are emollient and anthelmintic. Roots/ shoots are used to control excessive menstruation, blood purifier, digesting agent, piles. Some drugs of plant origin in conventional medical practice are not pure compounds but direct extracts or plant materials that have been suitably prepared and standardized. Establishment of the pharmacognostic profile of the leaves of *Amaranthus viridis* Linn will assist in standardization, which can guarantee quality, purity and identification of samples [1-2].



Fig 1: Morphology of leaves of *Amaranthus viridis* Linn

Materials and Methods

The plant of *Amaranthus viridis* Linn was collected in the month of January, 2017 from the house garden, Nasiyanur, Erode district, Tamil Nadu, India. The plant material was identified and authenticated by Dr. A Balasubramaniam, Research Consultant, ABS Botanical garden, Kaaripatti, Salem District, Tamil Nadu.

Phytochemical investigations

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins, phenols, cardiac glycosides, carbohydrates, alkaloids, Saponin, protein and amino acids, flavonoids, fats and oils, steroids^[3-4].

Pharmacognostical studies

Transverse section of leaves of (T.S) *Amaranthus viridis* L Fixation

Care was undertaken to select healthy plant and normal organs. The required sample of different was cut and removed from the plant and fixed in FAA (Formalin -5ml+ acetic acid 5ml+70% ethyl alcohol 90ml). After 24 hours of fixing the specimen was dehydrated with graded series of tertiary butyl alcohol^[5]. Infiltration of specimen was carried out by gradual addition of paraffin wax (melting point 58-60 °C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the hand and dewaxing of section was customary procedure^[6]. The sections were stained with toluidine blue. Since toluidine blue is polychromatic stain. The staining results were remarkable good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose wall, blue to the lignified cells, dark green to suberin, violet to mucilage, blue to protein bodies etc. Where ever necessary sections were also stained with safranin and IKI (for starch)^[7]. For studying the stomatal morphology, venation pattern and trichome distribution, Para dermal section (section taken parallel to the surface of leaf) as well as clearing of leaf with the 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffery's maceration fluid were prepared. Glycerin mounted temporary preparation were made for macerated/cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured.

Photomicrograph

Microscopic descriptions of tissues are supplemented with micrographs where ever necessary. Photographs of different magnifications were taken in Nikon lab photo 2 microscope units. For normal observations bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under the polarized light they appear bright against dark background. Magnifications of the figure are indicated by the scale bars^[8].

Analytical parameters

Ash values, extractive value, loss on drying were used for the study of physical properties.

Ash value

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore, an important parameter for purpose of evaluation of crude drugs. In certain drugs, the percentage variation of

the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. The ash value can be determined three different methods to measure the total ash, the acid insoluble ash and water soluble ash.

Determination of total ash value

Weigh accurately about 5gm of air dried powdered drug was taken in a tarred silica crucible and incinerated by gradually increasing the temperature to make it dull red until free from carbon cooled weighed then calculated the percentage of total ash with reference to air dried drug.

Determination in of acid insoluble ash value

Boil the total ash with 25ml of 2M HCl for 5 minutes, collect the insoluble matter in a gooch crucible or on an ash less filter paper and wash with hot water, ignite then cool in a desiccators and weigh. Calculate the percentage of acid insoluble ash with reference to the air dried drug.

Determination of water soluble ash value

Water soluble ash is that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drugs of incorrect preparation.

To the crucible containing the total ash, add 25ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450 °C. Subtract the weight residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg/g of the air dried material.

Determination of sulphated ash value

Heat silica of platinum crucible to redness for 10 minutes; allow cooling in desiccators and weighing. Unless otherwise specified in the individual monograph, transfer to the crucible 1 gm of the substance under examination and weigh the crucible and the contents accurately. Ignite, gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of H₂SO₄ heat gently until the white fumes are no longer evolved and ignite at 800± 25 °C until all black particles have disappeared. Conducted the ignition in a place protected from air current.

Extractive values

This method is to determine the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. It is employed for that material for which no chemical or biological assay.

Determination of alcohol soluble extractive value

5gm of air dried and coarsely powdered drug has to be macerated with 100ml of ethanol of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of ethanol. Evaporate 25 ml of filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105 °C and weigh. The percentage of ethanol soluble extractive value with reference to the air dried drug has to be calculated.

Determination of water soluble extractive value

The water soluble extractive value plays an important role for the evaluation of crude drugs. 5gm of the air dried and coarsely powdered drug as to be macerated with 100 ml of

water of the specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing for 18 hours. Thereafter, filter rapidly taking precautions against loss of water. Evaporate 25 ml of filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105 °C and weigh. The percentage of water soluble extractive value with reference to the air dried drug has to be calculated.

Loss on drying

Loss on drying is the loss of mass expressed as percentage w/w. It determines both water and volatile matter in the crude drug. It can be carried out either by heating at 100 °C -105 °C or in desiccators over phosphorus pent oxide under atmospheric or reduced pressure at room temperature for specified period of time.

Procedure

About 2 gm of powdered drug was taken in a tarred porcelain dish which was previously dried at 105 °C in hot air oven to constant weight and then weighed. Percentage of loss drying with reference to the air dried substance was calculated [9-10].

Results and discussion

Table 1: Data showing colour, Consistency and yields of methanolic extract of powdered leaf of *Amaranthus viridis* Linn.

Extract	Colour	Consistency	% Yield (W/W)
Methanolic extract	Dark green	Sticky mass	12 % (w/w)

Table 2: Results of the phytochemical constituents of Methanolic Extract of *Amaranthus viridis* L.

Phytoconstituents	Methanol
Carbohydrates	+
Fixed oils and fats	-
Protein and amino acid	+
Saponins	+
Steroids	+
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Gum and mucilage	-
Triterpenoids	+
Phenolic compounds	+

(+) Presence (-) Absence

Transverse section (T.S) of leaves of *Amaranthus viridis* L

T.S of petiole of leaves *A. viridis* L

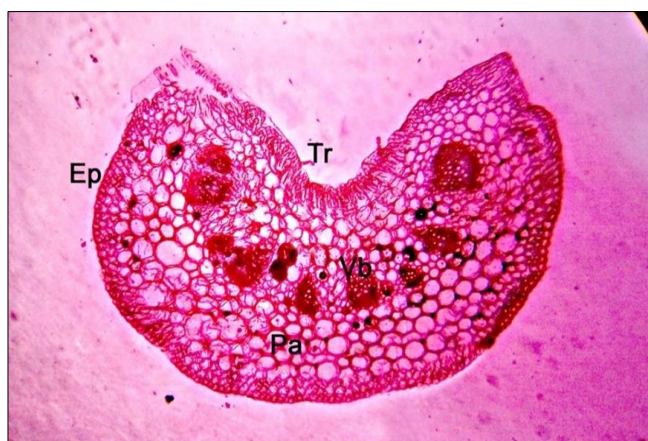


Fig 1: T.S of petiole of leaves of *Amaranthus viridis* L (Tr: Trichome, Ep: Epidermis, Pa: Parenchyma, Vb: Vascular bundle)

Description

Epidermis

The outer most layer of the petiole is epidermis. This is a single layer of parenchymal cells with anticlinal cell wall, almost circular, covered by cuticle. The stomata and trichomes are very rarely found. Tannins are found as a cell inclusion.

Cortex

Cortex is made up of polygonal, collenchymatous parenchyma, compactly packed, no intercellular spaces, heavy in cellulose, thick walled.

Vascular bundle

Numerous vascular strands are seen in the middle portion of petiole. They are bicollateral covered by pericyclic fibre which made up of lignified sclerenchyma cells. Xylem appears as round cells with high lignin content. Phloem is non-lignified surrounding the xylem.

T.S of midrib leaves of *Amaranthus viridis* L

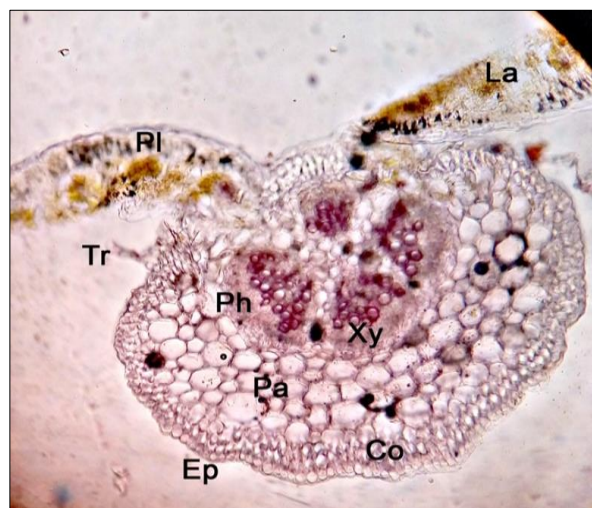


Fig 2: T.S of midrib of leaves of *Amaranthus viridis* L (Tr: Trichome, Ep: Epidermis, Pa: Parenchyma, Co: Collenchyma, Xy: Xylem, Ph: Phloem, La: Lamina, Pl: Palisade)

The T.S of midrib shows the following characters

Epidermis

It is a single layer of quadrangular parenchymal cell with wavy anticlinal cell walls, covered by cuticle externally. The stomatal pores are seen occasionally. The trichomes are both covering and glandular type.

Collenchyma

Two to three rows of thick walled, cellulosic collenchyma cells are present both upper and lower epidermis giving mechanical strength to the inner organs.

Parenchyma

Polygonal, thick walled, different sized parenchymal cells are seen in the cortical layer often filled with tannins and starch.

Vascular bundle

Vascular bundle is located exactly in the middle. Made up of four round vascular bundle consist of lignified xylem, non-lignified phloem surrounded by lignified sclerenchymatous fibres.

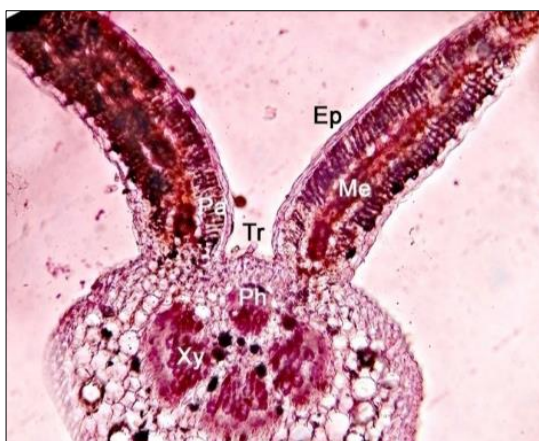
T.S of lamina of the leaves of *Amaranthus viridis* L.

Fig 3: T.S of the lamina of the leaf of *Amaranthus viridis* L (Tr: Trichome, Ep: Epidermis, Pa: Palisade Parenchyma, Xy: Xylem, Ph: Phloem, Me: Mesophyll)

Type of the leaf

Isobilateral. Both upper and lower epidermis are consists of same cellular arrangement.

Upper epidermis

Single layer of quadrangular parenchyma cells, anticlinal walls, cuticularized, showing covering and glandular trichomes and stomatal pores occasionally.

Palisade parenchyma

Palisade is a single layer of longitudinally elongated, compactly arranged parenchymal cells. Rich in chlorophyll pigments. They are not continuous in the midrib region. They are replaced by the presence of collenchyma cells in midrib region.

Mesophyll

Mesophyll is spongy in nature. In-between the palisade parenchyma, three to four rows of spongy parenchyma, thin walled, leaving large inter cellular spaces have been in the mesophyll region. The cells are consist cell inclusion as calcium oxalate crystals and starch grains.

Lower palisade

Both upper and lower palisade layers are same. Lower epidermis: same as upper epidermis. The numbers of stomatal pores are more in lower epidermis.

Trichomes

Covering trichomes: multicellular, uniseriate without branches, blunt at apex.

Glandular trichomes: unicellular body and unicellular head.

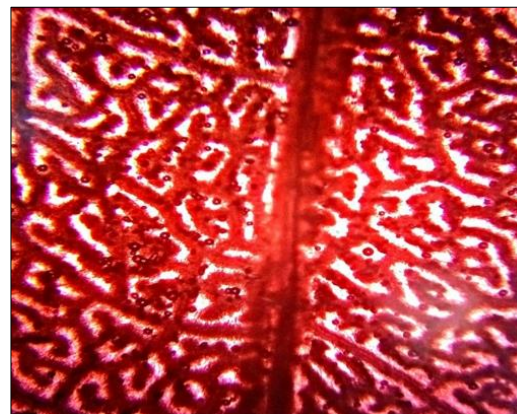
Venation pattern of leaves of *Amaranthus viridis* L

Fig 4: venation pattern of leaves of *Amaranthus viridis* L

Description

After clearing with clearing reagent, the leaf shows pinnate venation pattern. It shows the present of lateral veins, vein lets, vein islets and vein terminations. It also shows the presence of tannins and calcium salt deposition on the cell wall.

Analytical parameters

The analytical parameter were investigated and reported as total ash value (06.3% w/w), Water soluble ash value (1.20% w/w), Acid insoluble ash value (2.60%), Sulphated ash value (2.90% w/w), Water soluble extractive value (12.75% w/w), Alcohol soluble extractive value (12.60% w/w), Loss on drying (8.90% w/w). The above studies were enabled to identify the plant material for future investigation and form an important aspect of drug studies.

Table 3: The results were in the following table: Data for ash values for powdered leaves of *Amaranthus viridis* L

Physical parameters (Ash values)	Percentage (W/W)
Total ash value	06.3%
Water soluble ash	1.20%
Acid soluble ash	2.60%
Sulphated ash	2.90%

Extractive value and loss of drying

Table 3: Data for extractive value and loss on drying of powdered leaf of *Amaranthus viridis* Linn

Analytical parameters	Percentage (W/W)
Alcohol soluble Extractive Value	12.6%
Water soluble Extractive Value	12.75%
Moisture content	8.90%

Conclusion

Amaranthus viridis L is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. *Amaranthus viridis* L is a plant that has been confused with other species

due to their relative similarities. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. The micro morphological features of the leaf described, distinguishes it from other members of the general. These parameters could be useful in the preparation of the herbal section of Indian Herbal Pharmacopoeia.

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