



Anatomical and Histological Study of Stem, Root and Leaf of the Medicinal Plant *Amaranthus spinosus* Linn.

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Abstract

The transverse section of stem, root and leaf of *Amaranthus spinosus* Linn. was done with the help of sharp blade and double staining. The anatomy of stem and roots showed cellular differentiation. Both the stem and root showed secondary growth. In stem, the vascular bundle pattern is conjoint, collateral and endarch type; whereas root showed conjoint, collateral and exarch type of vascular bundle. Leaf anatomy showed kranz mesophyll. Endodermal wall is covered with casperian strips. The stomata (S) occurred on both adaxial and abaxial leaf surfaces. The stomata were found to be anomocytic type. Powdered drug, treated with different chemicals and its extracts with different solvent showed colour changes when illuminated with UV light.

Keywords: *Amaranthus spinosus*, secondary xylem, conjunctive tissue, kranz anatomy

Introduction

Stem of *Amaranthus spinosus* Linn are terete or obtusely angular, glabrous or slightly pubescent, green. The leaves alternate and are simple without stipules; petiole is approximately as long as the leaf-blade. The blade shape is ovate-lanceolate to rhomboid, acute and often slightly decurrent at base, obtuse, rounded or slightly retuse and often short mucronate at apex, entire, glabrous or slightly pubescent on veins when young. The inflorescence consists of dense clusters, lower ones are axillary, higher ones often collected in an axillary and terminal spike which is often branched in its lower part. Its pharmacognostic features are as follows: flowers are unisexual, solitary in the axil of a bract, subtended by 2 bracteoles; bracts and bracteoles scarious, mucronate from a broad base, shorter or as long as the perianth. Male flowers are usually arranged in a terminal spike above the base of the inflorescence (Fig. 1). Sepals are green, tepals 5 or in male flowers it is often 3. Tepals are free, subequal, ovate-oblong to oblong-spatulate, up to 2.5 mm long, very convex, membranous, with transparent margins and green band. Male flowers are with 5 stamens about as long as tepals. Female flowers are with superior, oblong ovary, 1-celled, styles 2-3, ultimately recurved. The fruit is ovoid shaped with a short inflated neck below the style base, circumscissile a little below the middle or indehiscent. The seed is about 1 mm in diameter, shiny, compressed, black or brownish-black in colour. It is widely distributed in the roadside of our locality. Due to industrial development we are continuously facing problems of pollution. Small canopy of this plant absorb suspended particle of various pollutant and make our environment healthy and protect our self

free from the various adverse effect of pollutant. So the main objective of this work is to know histological and anatomical feature of the stem, root and leaf of *Amaranthus spinosus* Linn.

Material and Methods

Materials

The plant *Amaranthus spinosus* Linn was collected from district Purulia, West Bengal in the month of June-July 2012. It was identified by the Department of Botany, BB College, Asansol, West Bengal, India. The reagents or solvents like alcohol, chloral hydrate, chloroform, glycerin water, Safranin, light green, 1N NaOH, ethanol, HNO₃, NH₃ solution, HCl, H₂SO₄, picric acid solution, glacial acetic acid, petroleum ether (60-80°C), chloroform, ethyl acetate were purchased from Merck Specialities Pvt. Ltd., Mumbai, India except one or two.

Dissection of stem, root and leaf

With the help of 7 o'clock blade different transverse sections were obtained by cutting along the radial plane of a cylindrical portion of the stem and root and the leaf was dissected with the help of potato. All the fine dissections were kept in watch glass along with water.

Staining procedure

The fine, selected sections were stained and it would help to distinguish different tissue, cells or inclusions from one another by developing specific colours.¹ Two stains were used in this work. The cell wall (cutinized / lignified wall) took safranin colour;

whereas soft tissue took light green colour. The fine thin sections were allowed to immerse in 50% alcohol for 3-5 min. Then, they were allowed to dip in safranin for 3 min. The sections were then washed with 50% alcohol. After washing, they were then placed in 70%, 80% and 90% alcohol successively for 5 min each. After that, the sections were placed in light green for 2 min and then washed

with absolute alcohol. Finally, they were mounted and were observed under microscope and photographed (camera model-COOLP1*L3) with ($\times 400$) magnifications. The behavior of powder with various chemical reagents was also carried out according to the standard procedures described by Khandelwal⁴ and Harborne.⁵

Table 1. Fluorescence analysis²⁻³ of powder of *Amaranthus spinosus* Linn (whole plant) with various chemical reagents

Reagents	Visible Light	UV Light (254nm)
Powder as such	No change	No change
Powder + 1N NaOH	Pale Yellow	Colour less
Powder + 1N NaOH in ethanol	Pale Yellow	Colour less
Powder + Ethanol	Pale Yellow	Colour less
Powder + HNO ₃ + NH ₃ Solution	Brown	Colour less
Powder + 50% HNO ₃	Light Brown	Pale Brown
Powder + HCl	Light Green	Brownish Green

Table 2. Analysis of different solvent extracts of *Amaranthus spinosus* Linn.(whole plant) under UV Visible light

Extract	Observation	
	Visible Light	UV Light (254nm)
Petroleum ether (60-80°C)	Yellow	Orange Red
Chloroform	Dark Brown	Deep Reddish Orange
Ethyl acetate	Greenish Brown	Reddish Orange
Ethanol	Dark Green	Orange
Water	Yellowish Brown	Light Green

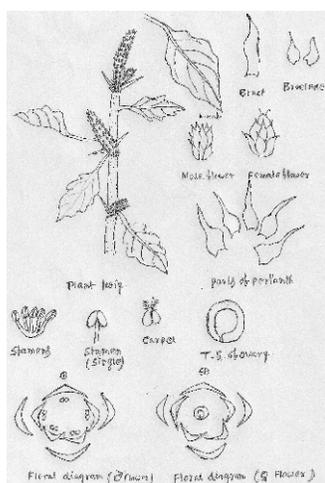


Fig. 1: Flowering twig and different parts of *Amaranthus spinosus* Linn

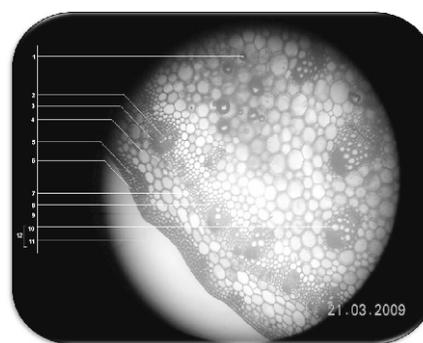


Fig. 2: Transverse section of *Amaranthus spinosus* Stem. Key: Oil Globules (1); Secondary Xylem (2); Secondary Phloem (3); Parenchyma (4); Hypodermis (Collenchyma) (5); Epidermis (6); Starch Sheath (7); Conjunctive Tissue (8); Pith (9); Primary Xylem (10); Primary phloem (11)

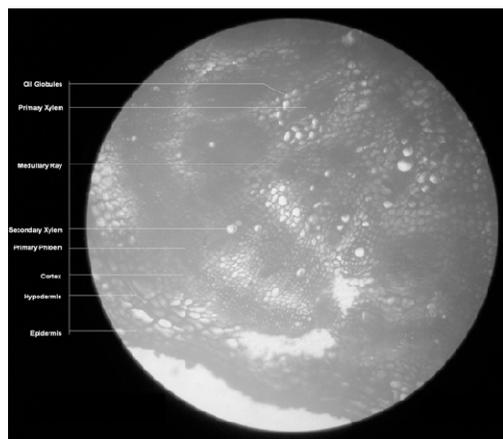


Fig. 3: Transverse section of *Amaranthus spinosus* root

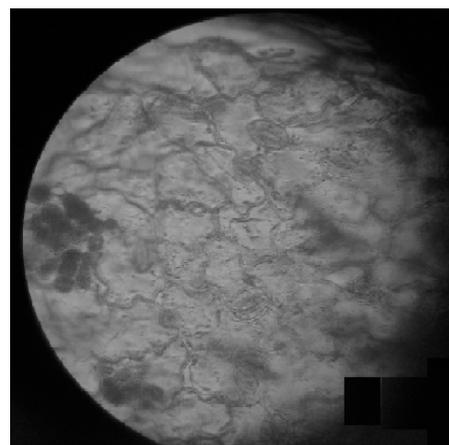


Fig.4: Peeling of leaf showing stomata

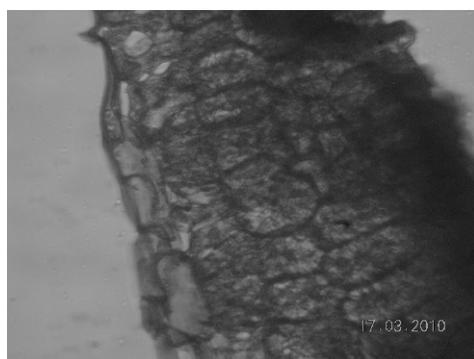


Fig. 5: Transverse section of leaf of *Amaranthus spinosus* showing Kranz anatomy

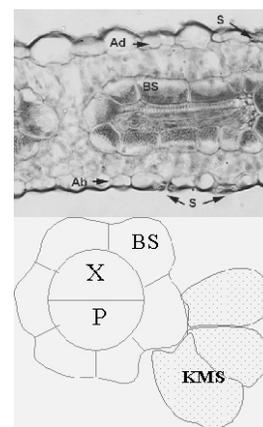


Fig. 6: Transverse section of leaf of *Amaranthus spinosus* showing Kranz anatomy

Results and Discussion

The powder characters of a drug are mainly used in the identification of the drug in the powder form (Table 1). The leaf powder was light green in colour, strong and characteristic in taste and on microscopical examination the powder showed anomocytic stomata, unicellular covering trichomes. Various solvent extract also showed colour changes under UV Light at 254nm (Table 2).

The outline of the stem and root section was almost circular (Fig. 2-3). Section showed the structures as follows. The epidermis was an outermost layer of barrel to rectangular cells. The cells were thickly cuticularised. A few stomata occurred in the epidermis and a few unicellular or multicellular hairs were also present. The cortex was multi-layered and differentiated into (a) collenchyma and (b) parenchyma. A few layered collenchymatous hypodermises follows epidermis. It was 3-5 layered deep. Parenchyma followed collenchymatous hypodermis and was few cells deep. The cells were spherical to oval. The cells might contain a few to many chloroplasts. In endodermis, a distinct endodermis with Casparian strip was present. A prominent starch sheath was present in its place. Pericycle was represented by a few sclerenchymatous cells

in the old stem. A large zone of vascular tissue lied just below the starch sheath. Starch sheath was followed by a large amount of conjunctive tissue in which secondary vascular bundles were embedded. Secondary phloem was situated just below the starch sheath. It was found in small groups. Two- layered ring of cambium separated secondary phloem from secondary xylem. Secondary xylem of secondary vascular bundle lied below the cambium. This secondary xylem was embedded in conjunctive tissue that appeared as a complete ring below the primary vascular bundles were called medullary bundles.⁶ The central part of the section had large parenchymatous pith. Cambial activity took place in these medullary bundles. Hence, a little amount of secondary phloem and secondary xylem were also present.⁷

Amaranthus spinosus Linn. is an example of a C4 dicotyledonous plant. The large bundle sheath cell, (BS) contains large chloroplasts. The mesophyll was described as radiate and was referred to as the Kranz mesophyll. These Kranz cells contain smaller chloroplasts than the underlying bundle sheath cell chloroplasts.

The simple diagram below the micrograph will help to understand

the relationships between the various layers making up "Kranz anatomy" (Fig. 5-6). The KMS (Kranz mesophyll) cells were intimately associated with the BS (bundle sheath) cells, and photoassimilated material (usually in the form of malate or aspartate) was passed through to the bundle sheath cells, via numerous plasmodesmata, which occurred at the interface between the bundle sheath and Kranz mesophyll. The root also showed secondary growth but here the vascular bundles were of conjoint, collateral, exarch type (Fig. 3). But in stem the vascular bundles were endarch type (Fig. 2). The stomata (S) occurred on both adaxial and abaxial leaf surfaces (Fig. 4). Leaves were dorsiventrally symmetrical. Columnar palisade cells were found in the upper epidermis and spongy parenchyma in the lower epidermis (Fig. 5). The stomata were found to be anomocytic type. The behavior of powder with various chemical reagent and preliminary chemical tests for various extracts were also carried out according to the standard procedures described by Khandelwal⁴ and Harborne.⁵

Conclusion

The large bundle sheath cell, (BS) contains large chloroplasts. The mesophyll tissue are of Kranz mesophyll. These Kranz cells contain smaller chloroplasts than the underlying bundle sheath cell chloroplasts. Stem showed secondary growth and the vascular bundle are of conjoint, collateral, endarch type. Root showed

secondary growth but here the vascular bundles are of conjoint, collateral, exarch type. The stomata (S) occurred on both adaxial and abaxial leaf surfaces. Leaves were dorsiventrally symmetrical. Columnar palisade cells were found in the upper epidermis and spongy parenchyma in the lower epidermis. The stomata were found to be anomocytic type.

References

1. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. *J Herb Med Toxicol* 2008; 2: 51-54.
2. Chase CR, Pratt RS. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J Am Pharmacol Ass-oc* 1949; 32:38.
3. Kokoshi CJ, Kokoshi RJ, Sharma FT. Fluorescence of powdered vegetable drug under ultraviolet radiation. *J Pharm Asses* 1958; 47: 715-717.
4. Khandelwal, KR (2005) *Practical pharmacognosy*, 13th ed., Pune: Nirali Prakashan, pp 146-148.
5. Harborne, JB (1973) *Phytochemical methods: a guide to modern techniques of plant analysis*, 3rd ed, Springer, pp 17-25
6. Gangulee H, Das C, Shankar K, Dutta C. *College Botany*. Vol.1, 6th ed., Kolkata: New Central book agency, 2007:390-392.
7. Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments*, 13th ed., Pune: Nirali Prakashan, 2005: 30-44.