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Full Length Research Paper

Pharmacognostic evaluation of the *Amaranthus viridis* L.

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The *Amaranthus viridis* Linn. (Family Amaranthaceae) plant was studied to determine the various parameters for pharmacognostical standards. The present investigation deals with the report on macro and microscopical, vein islet and vein termination numbers, palisade ratio, stomatal index (upper and lower surfaces of the leaf) and different chemical parameters have been determined. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

Key words: *Amaranthus viridis* Linn., pharmacognostical standards, macro and microscopical, chemical parameters.

INTRODUCTION

Amaranthus viridis Linn. is an annual herb, erect, 10 to 75 (-100) cm stem; slender, branched, angular, glabrous leaves; glabrous, long petiolate, 10 cm, lamina deltoid-ovate to rhomboid-oblong, 2 to 7 × 1.5 to 5.5 cm flowers; green, axillary or terminal, often paniculate spikes, 2.5 to 12 cm long and 25 mm wide. Bracts and bracteoles ovate to lanceolate-ovate, whitish, pale or reddish awn, bracteoles shorter than the perianth (1 mm); Perianth, male flowers, oblong-oval, acute, concave, 1.5 mm, female flowers narrowly oblong to narrowly spathulate, finally 1.25 to 1.75 mm, midrib green and thickened above. Stigmas 2 to 3, short, erect. Capsule subglobose, 1.25 to 1.5 mm. Seed, 1 to 1.25 mm, round, compressed, dark brown to black, reticulate. Flowering summer-fall (Ali and Qaiser (eds) 1995-2004). A decoction of the entire plant is used to stop dysentery and inflammation (Duke and Ayensu, 1985). The plant is antidiabetic, antihyperlipidemic and antioxidant (Ashok et al., 2010). The plant has antiproliferative and antifungal lectin (Kaur et al., 2006). The plant is emollient and vermifuge (Duke and Ayensu, 1985; Chopra et al., 1986). The root juice is used to treat inflammation during urination and constipation (Manandhar, 2002). The process of standardization can be achieved by stepwise pharmacognostic studies (Ozarkar, 2005). These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics (Anonymous, 1998). However, *A. viridis* is a common plant in certain parts of Asia especially Pakistan, where it is consumed as a leafy vegetable but there is very less information is available about the pharmacognostic parameters of this plant and therefore study is designed for pharmacognostical evaluation of *A. viridis* aiming towards standardization and correct identification of this species and differentiate it from the other species. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of *A. viridis* and microscopical characteristics of powdered plant specie.

MATERIALS AND METHODS

The first step in standardization of herbal drugs is the correct identification of plant macroscopic and microscopic characters. The fresh specimens of the plants were collected from the Department of Botany, University of Peshawar, Pakistan. The taxonomic identity of the plant was confirmed by Department of Botany Peshawar,

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Figure 1. *A. viridis* L.

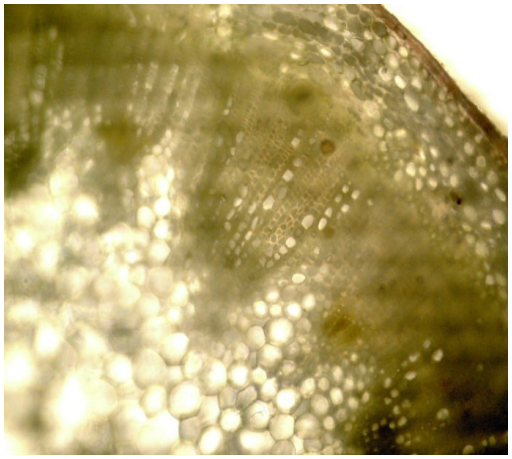

Figure 2. Transverse section of stem shows xylem and phloem.

University, Pakistan. A voucher specimen has been deposited in department herbarium. The specimen was cleaned, washed and air dried for 15 days and was used for different tests, that is, microchemical tests. These entire specimens were ground with the help of electric grinder and were preserved in airtight bottles to combat climatic conditions and moisture. Some fresh specimens were used to study morphological characters and anatomical parameters.

Macroscopy

The following macroscopic characters for the fresh parts of plant were noted: Size and shape, colour, surfaces, venation, the apex, margin, base, lamina, texture, odour and taste (Wallis, 1985; Evans, 2002).

Microscopy

The anatomies of the root, stem and leaf were determined by a standard method (Wallis, 1985; Evans, 2002). The outer epidermal membranous layer of leaf (in fragments) were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence/absence of epidermal cells and stomata (type and distribution) were observed. The transverse sections of the fresh leaf, stem and root as well as a small quantity of the powdered plant were also cleared, mounted and observed under a compound microscope (Clark, 1960; Bokhari, 1971; Cotton, 1974; African Pharmacopoeia, 1986; Subrahmanyam, 1996).

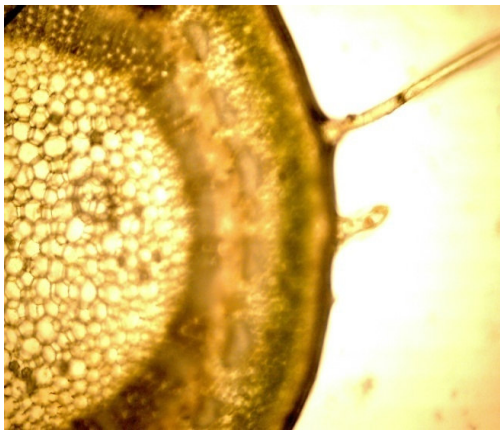

Figure 3. Transverse section of root show pericycle, cortex etc.

Chemomicroscopic examination

Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques (Evans, 2002).

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins, cardiac glycosides, alkaloids, saponins, anthracene derivatives and cyanogenetic glycosides (Johansen, 1940; Brain and Turner, 1975; Ciulei, 1981; Harborne, 1992; Evans, 2002).

Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein – islet number and veinlet termination number were carried out on epidermal peelings (British Pharmacopoeia, 1980).

RESULTS AND DISCUSSION

A. viridis 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 (Figures 1-5). *A. viridis* is a plant that has

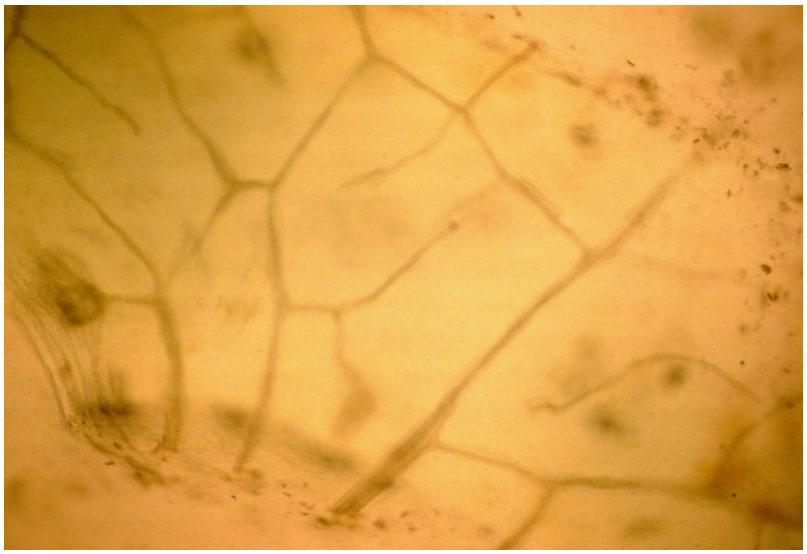

Figure 4. Lower side of leaf vein islet number and vein termination number.

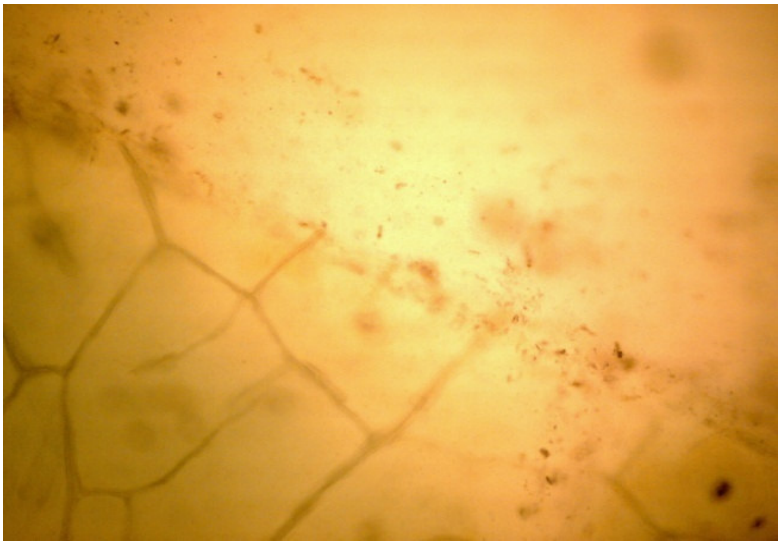

Figure 5. Upper side of leaf vein islet number and vein termination number.

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 macro – and micro – morphological features of the plant described, distinguishes it from other members of the genera. Chemomicroscopy, numerical data and quantitative plant microscopy are parameters that are unique to the plant and are required in its standardization.

Colour of the upper surface of the leaf is dark green and that of the lower surface is light green in fresh while in dry form both surfaces are light green in colour. Venation of the leaf was reticulate and unicostate. In both fresh and dry forms of the leaf; shape was cordate, composition was simple, margin was entire and apex was obtuse. In both fresh and dry forms of the stem: Kind was herbaceous, colour was light green, shape was cylindrical, direction of the growth was upright, fracture was fiberous, surface was smooth and phyllotaxis was opposite while odour was irritating in fresh and indistinct in dry form. In both fresh and dry forms of the root, the colour was whitish, shape was cylindrical, rootlets were present, direction of growth was horizontally downward, fracture was fibrous and texture was smooth while odour was pungent in fresh and indistinct in dry form (Table 1).

Leaf epidermal cells, both sides with polyhedral to hexagonal shaped of smooth walls. Size of epidermal cells; adaxail -137 × 45 µm. Stomata of anisocytic to

Table 1. Macroscopical features of the different parts of *A. viridis* L.

Plant part	Parameter	Fresh plant	Dry plant
Leaf	Colour	Upper surface dark green; lower surface light green	Both surface light green
	Composition	Simple	Simple
	Venation	Reticulate; unicostate	Reticulate; unicostate
	Margin	Entire	Entire
	Apex	Obtuse	Obtuse
	Shape of leaf	Cordate	Cordate
Stem	Colour	Light green	Light green
	Odour	Irritating	Indistinct
	Shape	Cylindrical	Cylindrical
	Phyllotaxy	Opposite	Opposite
	Kind	Herbaceous	Herbaceous
	Direction of growth	Upward	Upward
	Fracture	Fiberous	Fiberous
	Texture	Smooth	Smooth
Root	Colour	Whitish	Whitish
	Odour	Pungent	Indistinct
	Shape	Cylindrical	Cylindrical
	Rootlets	Present	Present
	Direction of growth	Horizontal downward	Horizontal downward
	Fracture	Fiberous	Fiberous
	Texture	Smooth	Smooth
	Composition	Simple	Simple
	Venation	Reticulate; unicostate	Reticulate; unicostate
	Margin	Entire	Entire
	Apex	Obtuse	Obtuse
	Shape of leaf	Cordate	Cordate

staurocytic type, with the length of 85 µm aperture size 12 µm. Epidermis of the stem is spherical in shape and is compactly packed. The mean length of the epidermis cells, cortical tissue, endodermis, pericycle, xylem, phloem, pith and parenchymatous cells was found to be 25, 13, 16, 15, 29, 20, 14 and 18 µm, respectively while the mean width was found 15, 7, 13, 11, 14, 12, 10 and 12 µm, respectively. Epidermis of the root is rectangular in shape and is compactly packed. The mean length of the epidermis cells, cortical tissue, endodermis, pericycle, xylem, phloem, pith and parenchymatous cells was found to be 34, 25, 19, 10, 14, 18, 34 and 13, respectively while the mean width was found 12, 18, 10, 5, 11, 12, 18 and 9 µm, respectively (Table 2).

Vein termination ranges from 44.65 t0 57.25, vein islet number ranges from 14.56 to 23.57 and the palisade ration ranges from15.62 to 24.42. Stomatal index of the upper surface of leaf is 21.25 to 24.62 and of the lower surface of the leaf of the plant 42.54 to 43.47 (Table 3). Alkaloids (Alk), saponins (Sap), starch (Sta), fat, protein (Pro) and cellulose (Cel) were present in all parts of the plant. Mucilage (Muc) and calcium oxalate (Cao) were present in stem only and were absent from other parts of the plant. Anthraquinon derivatives (Anth) and lignin (Lig) were present in root and stem and were absent from leaf and flower of the plant (Table 4). Yadav et al. (2007) reported flavonoids, saponins, steroids, alkaloids, carbohydrates and proteins in *Chenopodium album* Linn. root. Tannin was absent from all parts of the plant. Cutin was present in stem and leaf and was absent from root and flower. Badami et al. (2007) reported alkaloids, carbohydrates, proteins and amino acids, steroids, glycosides, saponins, tannins and fixed oils in *Caesalpinia sappan*.

A. viridis is a plant which is known to have some ethno pharmacological activities and is being well researched on. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant. These parameters which are being reported for the first time could also be useful in the preparation of the herbal section of the proposed Pakistani Pharmacopoeia. Any crude drug which is

Table 2. Anatomical features of the root and stem of the *A. viridis* L.

Plant cell	Value	Root		Stem	
		Length (µm)	Width (µm)	Length (µm)	Width (µm)
Epidermis	Minimum	25	10	22	14
	Maximum	43	16	30	18
	Mean	34	12	25	15
Cortex	Minimum	20	15	10	05
	Maximum	28	21	17	09
	Mean	25	18	13	07
Enodermis	Minimum	13	09	19	11
	Maximum	24	12	12	16
	Mean	19	10	16	13
Pericycle	Minimum	07	04	12	09
	Maximum	12	07	19	13
	Mean	10	05	15	11
Xylem	Minimum	12	09	26	12
	Maximum	19	14	34	19
	Mean	14	11	29	14
Phloem	Minimum	15	10	18	10
	Maximum	21	14	25	16
	Mean	18	12	20	12
Pith	Minimum	27	16	12	08
	Maximum	38	22	18	14
	Mean	34	18	14	10
Parenchyma	Minimum	10	07	15	10
	Maximum	17	12	21	15
	Mean	13	09	18	12

Table 3. Microscopic characteristics of the *A. viridis* L. Leaf.

S/No.	Parameter	Value
1	Vein Islet number	14.56 - 23.57/mm^2
2	Vein termination number	44.65 - 47.25/mm^2
3	Palisade ratio	15.62 - 17.42/mm^2
4	Stomatal index (Upper surface)	21.25 - 24.62
5	Stomatal index (Lower surface)	42.54 - 43.47

Table 4. Microchemical screening tests of the different parts of *A. viridis* L.

Plant part	Alk	Muc	Anth	Cao	Sap	Tan	Sta	Fat	Pro	Lig	Cut	Cel
Flower	+	-	-	-	+	-	+	+	+	-	-	+
Leaf	+	-	-	-	+	-	+	+	+	-	+	+
Stem	+	+	+	+	+	-	+	+	+	+	+	+
Root	+	-	+	-	+	-	+	+	+	+	-	+

+: Positive test; - : Negative test; Alk: Alkaloids; Sap: Saponins; Sta: Starch; Prp: Protein; Cel: Cellulose; Muc: Mucilage; Cao: Calcium oxalate; Anth: Anthraquinon derivatives, Lig: Lignin.

claimed to be *A. viridis* but whose characters significantly deviate from the accepted standard aforementioned would then be rejected either as contaminated, adulterated or down right fake.

REFERENCES

African Pharmacopoeia (1986). General methods for Analysis. OAU / STRC Scientific Publications, Lagos, 2(2): 0–5, 137–149, 223-237.

Ali SI, Qaiser M (eds) (1995-2004). Flora of Pakistan, Department of Botany, University of Karachi, Karachi, p. 12.

Anonymous (1998). Macroscopic and microscopic Examination: Quality Control Methods for Medicinal Plant Materials, WHO, Geneva.

Ashok KBS, Lakshman K, Jayaveea KN, Sheshadri SD, Saleemulla K, Thippeswamy BS, Veerapur VP (2010). Antidiabetic, antihyperlipidemic and antioxidant activities of methanolic extract of *Amaranthus viridis* Linn in alloxan induced diabetic rats. Exp. Toxicol. Pathol., Available online 18 July, 2010.

Badami S, Rai SR, Moorkoth S, Rajan S, Suresh B (2007). Pharmacognostic evaluation of *Caesalpinia sappan*. Hamdard Medicus, 50(1): 103–108.

Bokhari MH (1971). Morphology and Taxonomic Significance of Sclereids in *Limonium*. Notes. R. Bot. Gbn. Edin. 30: 43-53.

Brain KR, Turner TD (1975). Practical evaluation of phytopharmaceuticals. Wright – Scientechnica, Bristol. 1st Ed., p. 144.

British Pharmacopoeia (1980). Appendix XI. Her Majesty's Stationery Office,London, A108, 11: A113.

Chopra RN, Nayar SL, Chopra IC (1986). Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, CSIR Publications, New Delhi.

Ciulei I (1981). Methodology for analysis of vegetable drugs. United Nations Industrial Development Organisation. Romania, pp. 17-25.

Clark J (1960). Preparation of leaf epidermis for topographic study. Stain Technol., 33: 35-39.

Cotton R (1974). Cytotaxonomy of the genus *Vulpia*. *Ph. D Thesis*, University of Manchester, USA.

Duke JA, Ayensu ES (1985). Medicinal Plants of China Reference Publications, Inc., pp. 20-24.

Evans WC (2002). Trease and Evans Pharmacognosy. 13th ed. Bailliere Tindall, London, pp. 654–656.

Harborne JB (1992). Phytochemical methods. A guide to modern technique of plant analysis. Chapman and Hill, London, p. 279.

Johansen DA (1940). Plant microtechnique. McGraw Hill Book Company. New York and London, pp. 189–202.

Kaur N, Dhuna V, Kamboj SS, Agrewala JN, Singh J (2006). A novel antiproliferative and antifungal lectin from *Amaranthus viridis* Linn seeds. Protein Pept. Lett., 13(9): 897-905.

Manandhar NP (2002). Plants and People of Nepal Timber Press. Oregon, p. 6.

Ozarkar KR (2005). Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* Linn. and *Valeriana wallichi* DC using mouse model. Ph.D. Thesis,University of Mumbai, Mumbai.

Subrahmanyam NS (1996). Labortary Manual of Plant Taxonomy, Vikas Publishing house pvt., Ltd., New Delhi, India, pp. 153–156.

Wallis TE (1985). Textbook of Pharmacognosy, 5th Edition. CBS Publishers and Distributors, 485 Jain Bhawan, Shahdara Delhi, pp. 252-253.

Yadav N, Vasudeva N, Sharma SK, Singh S (2007). Pharmacognostic and phytochemical studies on *Chenopodium album* Linn., root. Hamdard Medicus, 50(1): 95–102.