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PHARAMCOGNOSTICAL AND PHYSICOCHEMICAL CHARACTERIZATION OF *AMARANTHUS GRAECIZANS* SUBSP. *SILVESTRIS*: AN ANATOMICAL PERSPECTIVE

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Abstract

Amaranthus graecizans subsp. *silvestris* (Vill.) Brenan, a medicinal herb belongs to family Amranthaceae. Pharamcognostical and physicochemical characterization of *A. graecizans* subsp. *silvestris* which included; macro and microscopic evaluation, phytochemical and physicochemical analysis of leaf, stem, root, fruit and seeds was investigated. Transverse sections of leaf, stem and root showed the arrangement of different cells, certain tissues that will serve as diagnostic characters to standardize this plant. The powder microscopy of leaf, stem, root, fruit and seed depicted various microscopic structures including; fibres, vessels, tracheids, oil cells, starch granules, cortical cells, cork cells, phloem, collenchyma and parenchyma tissues etc. In fluorescence analysis different colors were seen when extracts were exposed to ordinary and UV light. Phytochemical screening of methanolic extract of whole herb exhibited the occurrence of saponins, tannins, carbohydrates, flavonoids, cardiac glycosides, sterols, lipids and alkaloids. Physicochemical analysis i.e. extractive values and ash values were calculated to strengthen standardization process. These findings and estimations will help in characterization, verification and quality maintenance of *A. graecizans* subsp. *silvestris*.

Key words: *Amaranthus graecizans* subsp. *silvestris* (Vill.) Brenan, Physicochemical, Pharmacognostic, Phytochemical

Introduction

Amid the most recent couple of decades, conventional frameworks of pharmaceutical have accomplished more worldwide significance due to its adaptable beneficial effects on human health. In various developing countries, a great number of population relies on medicinal plants to fulfill basic and primary healthcare needs, therefore phytomedicines have frequently gained more popularity for their historical and traditional reasons (Aburjai *et al*., 2007). Quality of these medicines relies on the proper authentication of the plant material utilized and among the standard procedures adopted to standardize the herbal material, pharmacognostic evaluations is one of the important tool. The initial authentication is carried out by macroscopic characterization of plant material. Due to close resemblance of different taxa within the genus, there are chances of adulteration during collection. This necessitates the need of plant material standardization including qualitative and quantitative estimations (Ishtiaq *et al.*, 2016).

Around 60–75 species belong to genus Amaranthus distributed all over the world (Talebi *et al*., 2016). Certain species of Amaranthaceae and Chenopodiaceae are medicinally important (Ajaib *et al.,* 2016) but difficult to identify. It is difficult to describe and characterize species of Amaranthus taxonomically because of close resemblance among species. Common similarities include the minute botanical characters among the species as well as intermediate forms (Jacobsen & Mujica, 2003). Species of Amaranthus have medicinal values and are reported to have certain biological actions. *Amaranthus graecizans* subsp. *silvestris*is one of the varieties reported in Pakistan (Townsend), which has been utilized as traditional remedy in the treatment of gonorrhea, inflammation, piles and exhibit mild antibacterial spectrum. The plant extracts have also revealed antioxidant activity that makes it a suitable agent for the treatment of various degenerative disorders (Koochak *et al.,* 2010; Arshad *et al*., 2011; Ishtiaq *et al*., 2014). The methanolic extract of the herb is also reported to possess hepatoprotective, analgesic, anti-inflammatory, *in vitro* protease and acetyl cholinesterase inhibitory action (Ishtiaq *et al.*, 2017a, 2017b).

In spite of being an important medicinal herb, yet no detailed guidelines are available to standardize the plant material. Hence, the present investigations encompass botanical and pharmacognostical analysis that are necessary to establish the quality of plant material by performing organoleptic and microscopic observation, physicochemical, fluorescence analysis and preliminary phytochemical screening of the *A. graecizans* subsp. *silvestris.*

Materials and Methods

Plant material: Fresh plant material was collected from Chakwal, authenticated from Department of Botany, Mirpur University of Sciences and Technology (MUST), Pakistan with voucher no. MUST. BOT. 5355.Plant parts *i.e.* leaves, stem, root, fruit and seeds were separated. All plant parts were dried under shade, were powdered and preserved in brown containers at dry place.

Chemicals and instruments: Analytical grade alcohol, methanol, chloroform, *n*-hexane, ethyl acetate, glacial acetic acid, sulphuric acid, sodium hydroxide, hydrochloric acid, potassium hydroxide, aniline and ferric chloride. All the chemicals were purchased from Sigma Aldrich, Germany. Distilled water and chemical reagents: Wagner's reagent, Dragendorff's reagent, Millon's reagent,

Molisch's reagent (distilled water and all the reagents were freshly prepared in the lab during phytochemical evaluations). Microscope (Labomed, UK) and Canon Powershot SX 220HS Japan.

Pharmacognostical evaluations

Macroscopic evaluation: All macroscopic evaluations were carried out on 5 samples of each part. The taxonomical description was made according to the related articles and the data given in books (Kokate *et al.*, 2006).

Microscopic evaluations

Transverse section cutting (T.S.): Fresh leaf, stem and root was immediately fixed for 24 hrs. in formalin: acetic acid: 70% alcohol (5:5:90). Sections were made by blade and razor method and were stained with safranin and fast greendye (Sylevester & Ruzin, 1994).

Powder study: The powdered drug was mixed with chloral hydrate (75%) solution for ease in microscopic observation. Slides of powdered plant parts were prepared according to the prescribed procedures (Ishtiaq *et al.*, 2018).

Fluorescence analysis: Fluorescence analysis of powdered plant parts were carried out by treating them with different reagents and then observed in ordinary light and UV light (short wavelength of 254nm and long wavelength of 366 nm) (Akbar *et al.*, 2014).

Phytochemical analysis: The phytochemical analysis was carried out according to the standard procedures (Aslam & Afridi, 2017).

Physicochemical analysis: Powdered samples were subjected to physicochemical analysis for their extractive values, and ash values (Harborne, 1998; Evans, 2003).

Results

Macroscopic features: *A. graecizans* subsp. *silvestris* (Vill.) Brenan is an annual herb, pubescent at distal parts (Fig. 1). It is smooth, slightly pale or reddish green in color. The height of plant ranges from 20–50cm, with erect and furrowed stem. Twigs are upward and single. The leaves are alternate and obtuse, rhombic or lanceolatein shape, long-petiolate. The margin is simple and sinuate. The leaf-blade of the main stem leaves is broadly to rhomboid-ovate or elliptic-ovate 3x1.5cm. Fruits are in the form of a capsule, rough, indehiscent, rupturing irregularly or with a circumscissile lid. Seeds are almost globose or orbicular, slightly reticulate, smooth, brown to black, but shiny. The dimension of seed is 1.3x1.1mm. Flowers are pistillate, greenish and unisexual, occurs in the form of clusters arranged axillary. Bracts and 2 bracteoles present. Perianth segments (3–5mm) very shortly mucronate, free at the base and membranous. Flowering period July to October (http://www.tela-botanica.org/bdtfx-nn-3962-synthese).

Fig. 1. Plant of *Amaranthus graecizan* ssubsp. *Silvestris* (Vill.) Brenan.

Microscopic characters through Transverse sections

Stem: The transverse section of stem revealed various structures. The diagnostic characters were; periderm showing narrowly packed cells, the collenchyma of the cortex, starch sheath, primary and secondary phloem, primary and secondary xylem, pith situated in the center which stored food granules and surrounded by vascular bundles.

Leaf: The transverse section of leaf revealed various structures. The diagnostic characters were; leaf epidermis, epidermal cells, palisade layer, trichomes, xylem, collenchyma and spongy mesophyll.

Root: The transverse section of root revealed different structures. The diagnostic characters were; periderm, cortical cells, vessels, metaxylem and protoxylem and sclerified cells.

Powder microscopy: The leaf powder was dark brown in color, having faint odor and a slightly bitter taste. The microscopic features of powder studied under compound microscope revealed various structures (Figs. D–H). The diagnostic characters were; leaf vein islets, oil glands, vein termination and vascular tissue, filiform fibers and epidermis, vessel with scalariform wall thickening, collenchyma and cortical cells, unlignified fibers with a single long cell.

The powder of root was light brown in color, having weak odor and unpleasant taste. The microscopic features of powder revealed various structures (Figs. I–K). The diagnostic characters were; cork cells which has suberized walls with underlying cortical cells, tracheid with scalariform wall, tracheid and vessels; vessel with annular thickening and perforation.

Fig. A. Transverse section of stem *A. graecizans* subsp. *silvestris* **a:** periderm **b:** vessels **c:** pericycle **d:** starch sheath **e:** interfacicular region **f:** crushed xylem **g:** primary phloem

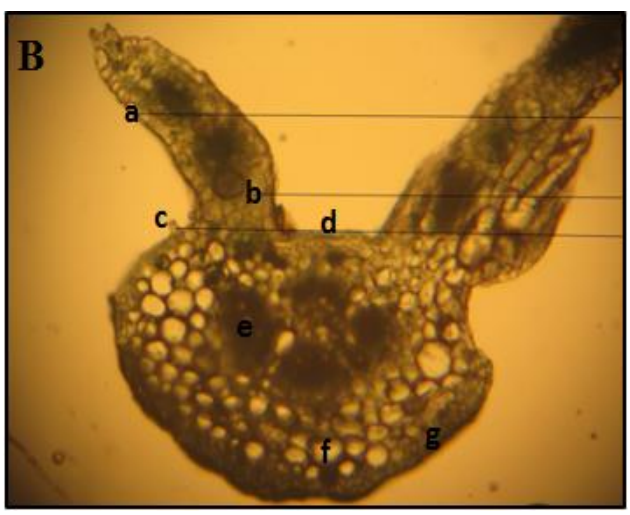


Fig. B. Transverse section of leaf *A. graecizans* subsp. *silvestris* **a:** epidermal cell **b:** palisade layer **c:** trichome **d:** epidermis **e:** xylem tissues **f:** spongy mesophyll **g:** collenchyma

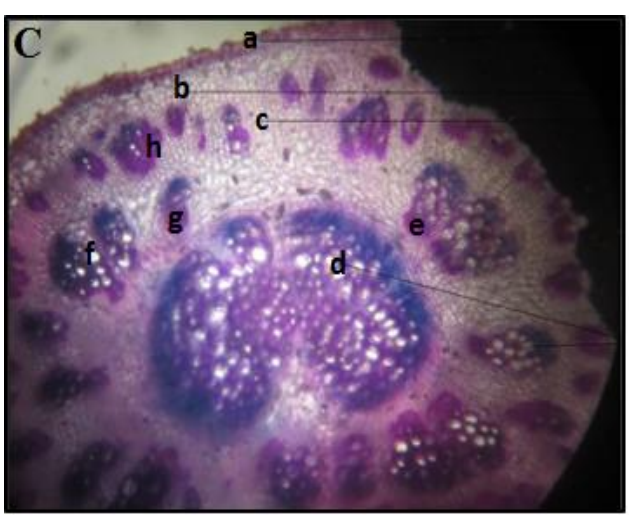


Fig. C. Transverse section of root of *A. graecizans* subsp. *silvestris* **a:** periderm **b:** cortical cells **c:** phloem tissue **d:** vessels **e:** protoxylem **f:** metaxylem **g:** sclerified cells

The powder of stem was pale brown in color with a unique odor and taste. The microscopic feature of powder revealed various structures (Figs. L–P). The diagnostic characters were; vessel, stem fiber, phloem, oil cells and starch granules.

The powder of fruit and seed was yellowish brown in color having specific smell and flavor. The microscopic feature of powder revealed various structures (Figs. Q–U). The diagnostic characters were; epicarp having middle stony layer, cork cells with suberized wall material, nucleated fiber with cytoplasmic content, parenchymatous cells and cortical cells having multilayers.

Fluorescence analysis: Fluorescent compounds give colors when exposed to short and long wavelength U.V light. The analysis showed various colors of the extracts under ordinary light, short wavelength (254nm) UV light, and long wavelength (366nm) UV light (Table 1).

Preliminary phytochemical analysis: The phytochemical screening of plant material mainly exhibited the occurrence of saponins, tannins, carbohydrates, flavonoids, cardiac glycosides, sterols, lipids and alkaloids (Table 2).

Physicochemical analysis: The extractive values are an important part of physicochemical analysis and they are calculated to strengthen the standardization procedure. The evaluation was performed with ethanol and chloroform (Table 3).

The ash values is one of a type of quantitative test that discloses the presence of unwanted material which is included during collection of plant material. Total ash, acid soluble and water insoluble ash was determined (Table 4).

Discussion

Pharmacognostic studies are the easiest and the simplest method for the identification and authentication of the plant materials. These studies help to establish the standards to maintain the quality of plant medicinal material (Panda, 2004). Macroscopic along with the microscopic evaluations provide the comprehensive and detailed information that is helpful even for the authentication of closely related species of same genus (Thirumalai *et al*., 2013). Microscopic studies *i.e.* transverse sections and powder microscopy of different parts (leaves, stem, root, fruit and seed) of *A. graecizans* subsp. *silvestris* were performed to set authentication standards that later would be helpful in verification at the time of plant material collection. The stem and leaf both showed the presence of periderm (Figs. A & B). Periderm is defensive tissue that replaces the epidermis when the optional development dislodges the epidermis of primary plant body. It secures the plant against neurotic epithelial grips amid embryogenesis (Richardson *et al.*, 2014). Likewise, the collenchyma cells were also observed in the stem and leaf (Figs. B & C). Collenchyma tissues are responsible for the mechanical support and structure symmetry of the plant (Mauseth, 1988). They play an important role in flexural rigidity in certain plants during draught conditions (Caliaro *et al.*, 2013). In TS of leaf, palisade tissue, spongy mesophyll tissues and trichomes were also present. Palisade tissues, contain the choloroplasts in them, which is required for the process of photosynthesis (Vogelmann & Martin, 1993). On the other hand trichomes were among the main anatomical components of plants to be perceived by early microscopists.

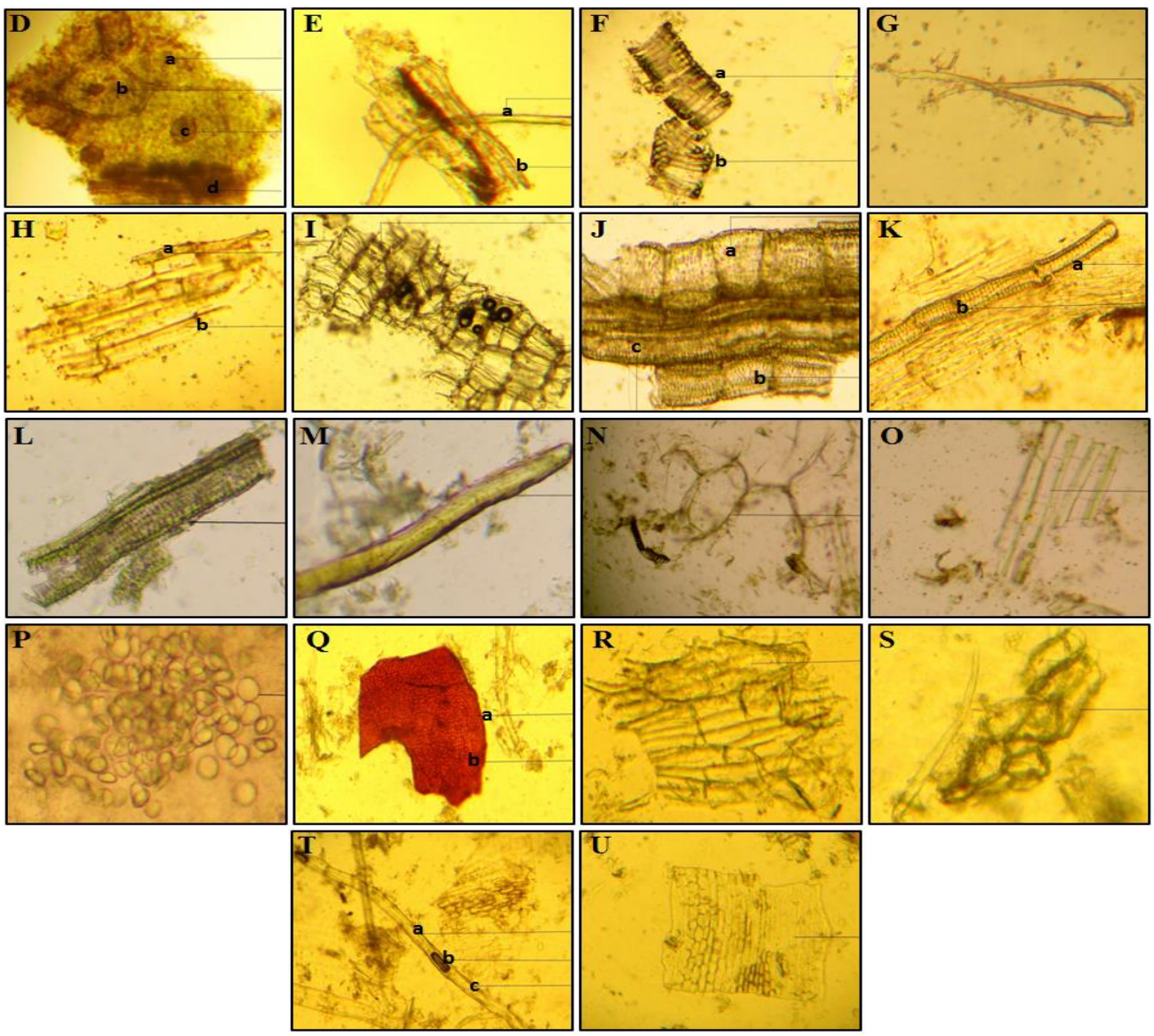
They play role in maintaining water balance, reflects the radiations, lowers the plant temperature and prevents water loss in the plants (Wagner *et al.,* 2004).

The powder microscopy of leaf, stem and powder showed the presence of certain common structures *viz.*, Fibres, cork cells, vessels, parenchymatous tissues *etc.* (Figs. D-U).Cork cells act as outer protective cellular layer, fibres are responsible for the mechanical strength, parenchyma function in storage and photosynthesis, while tracheids and vessels both boarder pitted or spiral are involved in the transport system of the plant (Mauseth, 1988; Sperry *et al.,* 2006). Starch granules were also present and observed during microscopy. This depicts the nutritional value of the herb in terms of carbohydrate content of the plant (Deatherage *et al.*, 1955).

UV fluorescence analysis is one of the simplest and finest method to find out the fluorescent compounds. In fluorescence analysis the dry powder was mixed with different chemicals and then exposed to short and long wavelength UV light. Different colors were observed (Table 1) confirming the presence of fluorescent compounds (Joshi, 2012).

The preliminary phytochemical screening of MeOH extract of whole plant exhibited the occurrence of carbohydrates tannins, flavonoids and sterols as major groups (Table 2). These phytochemicals are considered to be responsible for the various therapeutic effects that are ascribed to this plant material. The phytochemical groups were similar as reported by Ishtiaq *et al.*, 2014.

Physiochemical analysis adds further quantitative support to the plant authentication methodology. Extractive values plays a vital role in the plant material evaluation. A low extractive value is the indicative of addition of exhausted material (Table 3) while it is illustrated in Table 4 that the ash values reveals the unwanted material collected or remained attached with the plant during collection (Mukherjee, 2002; Khandelwal, 2008).



Figs. (D–U): **D) a:** vein termination **b:** vein islets **c:** oil gland **d:** vascular tissue. **E)** Filliform fibres **a:** fibre **b:** epidermis. **F)** Vessels with scalariform wall thickening **a:** lignin **b:** vessel. **G)** Lignified fibre. **H)a:** Collenchyma **b:** cortical cells. **I)a:** cork cells **b:** cortical cells. **J)** Primary xylem cells in the form of tracheids and vessels **a:** tracheid with scalariform wall **b:** tracheid **c:** vessels. **K)** Vessels with perforations **a:** vessels with annular thickening **b:** perforation. **L)** Stem vessels. **M)** Stem fibre. **N)** Stem oil cells. **O)** Stem phloem. **P)** Stem starch granules. **Q)** Seed **a:** seed coat **b:** stony layer. **R)** Parenchyma tissue. **S)** Cork cells. **T)** Seed fibre **a:** fibre **b:** nucleus **c:** cytoplasm. **U)** Cortical cells.

Table 1. Fluorescence analysis of whole herb, *A. graecizans* subsp. *silvestris*.

S. No.	Protocols	Inferences		
		Ordinary light	Short wave length 254 nm	Long wave length 366 nm
1.	Dry powder	Green	Dark green	Brown
2.	Powder + 5% NaOH	Pale Yellow	Dark green	Light green
3.	Powder + 50% H_2SO_4 in water	Light green	Dark green	Dark brown
4.	Powder + 5% $FeCl_3$	Yellowish brown	Dark green	Dark brown
5.	Powder + 50% HNO_3 in water	Yellow	Dark green	Dark brown
6.	Powder + Chloroform	Greenish yellow	Light green	Dark pink
7.	Powder + Water	Light yellow	Light green	Dirty green
8.	Powder + 66% H_2SO_4	Green	Dark green	Brown
9.	Powder + Aniline	Red	Black	Dark brown
10.	Powder + conc. KOH	Light brown	Dark green	Brown

Table 2. Preliminary phytochemical screening of whole herb, *A. graecizans* subsp. *silvestris*.

S. No.	Groups	Tests	Inferences
1.	Proteins	Millon's test	–
		Ninhydrin test	–
		Biuret test	–
2.	Carbohydrates	Barfoed's test	–
		Molisch's test	++
		Benedict's test	+
3.	Saponin	Foam test	+
		Bromine water test	+
4.	Tannins	Ferric chloride test	++
5.	Triterpenoids	Salkowaski test	–
		Liebermann's test	–
6.	Sterols	Salkowaski test	++
		Liebermann's test	++
7.	Cardioactive Glycosides	Bromine water test	+
		Keller-killani test	+
		Legal's test	+
8.	Flavonoids	Ferric Chloride test	++
		Alkaline reagent test	+
9.	Alkaloids	Mayer's Test	+
		Wagner's test	+
		Hager's test	+
		Dragendorff's test	++

"+" represents moderate presence, "++" strong presence and "–" represents absence

Table 3. Extractive values of whole herb, *A. graecizans* subsp. *silvestris*.

S. No.	Parameters	% Age yield ± (SEM)
1.	Ethanol	0.08 ± 0.003
2.	Chloroform	0.026 ± 0.02

Table 4. Ash values of whole herb, *A. graecizans* subsp. *silvestris*.

S. No.	Parameters	Values % (w/w)
1.	Total ash	2.07 ± 0.12
2.	Acid insoluble ash	0.40 ± 0.01
3.	Water soluble ash	0.19 ± 0.02

Conclusion

The present findings concerns with the pharmacognostical characterization and physicochemical properties of *Amaranthus graecizans* subsp. *silvestris* and the standardization of parameters like macroscopic and microscopic features of plant parts, phytochemical screening of the MeOH extract, fluorescence analysis for screening of fluorescent compounds. The ethanol extractive values were significant. The estimation of ash values positively impacted the crude drug standardization. The findings made in this work will be helpful in authentication and quality management of this plant material.

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