Pharmacognostic and Phytochemical Studies on *Amaranthus graecizans* L.

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In present paper an attempt has been made to find out the pharmacognostic, phytochemical and analytical characteristics of *Amaranthus graecizans,* an important medicinal plant being used traditionally for the treatment of various ailments. The important parameters studied are macroscopic characteristics, fluorescence behaviour under UV light, successive extractive values, total ash, acid insoluble ash, quantitative ash analysis for inorganic constituents and qualitative analysis of different extractives for organic chemical constituents. TLC, UV, TLC finger prints of different extractives and their analyses have also been performed.

Keywords: *Amaranthus graecizans,* Pharmacognosy, Phytochemistry, Standardization.

Introduction

The plant has edible leaves and is used as a pot-herb and vegetable and also as a fodder for cattle. In Mauritania the seeds are baked into thin cakes. The plant is used by traditional medicine practitioners for treatment of inflammatory conditions. The leaves are used as emollient; Crushed leaves are applied to scorpion sting and snake bites and irritating or itchy rashes. Whole plant used to treat generalized oedema and as a poultice for mastitis. This plant is an astringent and it is used externally as a gargle in ulcerated conditions of the mouth and throat and as a wash and poultice for ulcers and sores (Miller 1988, Miller 1996).

The plant elicited a significant analgesic activity; antidepressant activity (strong spasmolytic activity/anti-spasmodic action of the plant extract); antidiarrhoeal activity; cytoprotection activity tested against necrotizing agents and gastro protective activity against indomethacin. The plant extract on isolated guinea pig tracheal chain caused relaxation of Guinea pig tracheal chain (bronchodilatory activity); positive uterotropic activity and anti-stress activity as revealed by the swim endurance test.

Materials and Methods

The aerial parts of *Amaranthus graecizans* were collected from Liwa-Bu Sadain (Marghah), UAE. The specimen of the materials as specimen No. ZCHRTM-319, is preserved in the herbarium of the Zayed Herbal Research Centre, UAE for record. The stems and leaves were separated, dried under shade and processed using standard methods (Trease and Evans, 1996). The powdered materials were used for fluorescence analysis and successive extraction using Soxhlet extractor. Standard procedures from different pharmacopoeias were opted for physico-chemical studies (Anonymous, 1966; BHC, 1992; BHP, 1997; *Chinese Pharmacopoeia,* 1997). To develop the thin layer chromatograms, silica gel 60 F254 precoated glass plates (Merck, Darmastadt) were used. Qualitative analyses of the 10% alcoholic extract for different organic constituents were done employing usual methods (Trease and Evans, 1996). The ash of the powdered material was quantitatively analysed for inorganic chemical constituents by atomic absorption spectroscopy.

TLC Fingerprints of Different Extractives of AGA

Dissolved 0.05 g of petroleum ether (60-80°) and methanol extracts in 5 ml. of toluene and methanol respectively

and ultrasonicated for 15 minutes and decanted the supernatant liquid. With the help of a micro syringe 5 µl of each extractive in toluene and methanol were applied on silica gel film in circular compact spots. In every case TLC tank was allowed to saturate with the chromatography solvent, being used at room temperature for at least 30 minutes before placing the chromatoplate in it. TLC splitting patterns for these extractives were studied in different chromatography solvents.

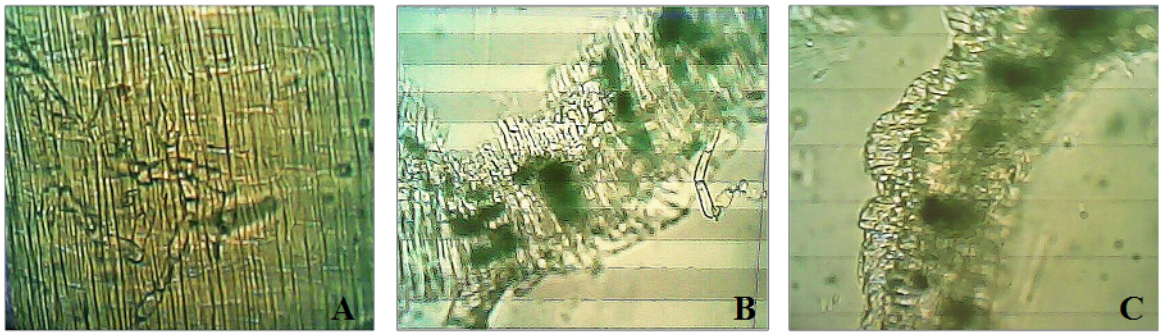
Results and Discussion

Microscopic Characteristics

The surface view of the leaf shows very intricate venation and cells embedding the vascular tissues are quite characteristic and they are oblong, of similar sizes and shapes and they are set adjacently in a continuous order. These oblong cells are devoid of calcium oxalate cluster crystals but other cells isolated by these characteristic are rich in these crystals in such a manner that each cell generally contains a comparatively large crystal. Elongated parenchyma cells exist alongside the main veins and other main branches bear uniseriate glandular trichomes with rounded heads. Unicellular covering trichomes are occasional. The ventral surface of the leaf (upper epidermis) contains a number of stomata-like structures whose cells are thicker than normal stomatal guard cells while the dorsal surface (lower epidermis) contains many small anomocytic oval stomata. A sectional view of the leaf exhibits its dorsiventral character. The upper epidermis is shown to consist of elongated compressed cells that bulge outwards into angular waves. The elongated epidermal cells at the margins contain smaller cluster crystals of calcium oxalate. The palisade cells underlying the upper epidermis are cylindrical, slender and compactly packed. The spongy mesophyll cells are oblong and they form many layers. The lower epidermal cells are polygonal with wavy cell walls.

The dried stems and branches are pale yellow in colour with a pinkish tint, glabrous with square cross-sections and prominent two opposite ridges. The branch is brittle and branching is at internodes.

A transverse section of the stem exhibits its square outline with prominent two opposite ridges. The epidermis consists of elongated parenchyma cells which are closely packed and it bears uniseriate covering trichomes. It is underlined by a layer of oblong cortical yellow-coloured parenchyma cells when viewed through a sectional view of the stem whereas they appear as square cells on surface view. These are underlined by a circle of unlignified fibres followed by many layers of compressed parenchyma cells with pitted, thick cell walls which are also surrounding a circle consisting of a number of layers of lignified yellow-coloured cells separated from the underlying vascular tissues by a few layers of thick-walled polygonal parenchyma cells. The xylem tracheids are narrow and they have pitted cell walls; the vessels are compactly packed together and they are mostly spirally thickened, while the xylem fibres are long and all xylem tracheids, vessels and fibres are lignified. Some xylem parenchyma cells contain small crystals of calcium oxalate. The pith is composed of many cells with different shapes but they are mostly circular and polygonal parenchyma cells.



A = TS of a portion of the leaf showing part of the epidermis, the cylindrical compactly packed palisade cells, the multi-layered spongy mesophyll tissues that are rich in calcium oxalate cluster crystals (dark areas) and the lower epidermis.

B = A surface view of the stem showing rectangular epidermal cells and some characteristic multicellular covering trichomes. The epidermis exhibits underlined square-shaped outer cortical parenchyma cells.

C = TS through a portion of the stem showing from the epidermis inwards: the epidermis with elongated parenchyma cells underlined by a layer of yellow coloured cortical parenchyma cells; a circle of unlignified fibres; compressed parenchyma cells with pitted walls; layers of lignified yellow coloured cells layers of thick -walled cells; vascular tissues (lignified); then wide pith zone.

TABLE 1
Behaviour of Powdered Materials with Some Chemical Reagents

Treatment	Observations
	AGA Powder
Powder triturated with water	Emulsion formed
Powder shaken with water	No frothing, bulk of the powder settles down
Powder treated with 5% aqueous NaOH	No change
Powder treated with 5% aqueous $FeCl_3$	Turns light brown
Powder treated with 66% aqueous H_2SO_4	No change
Powder pressed between two filter paper for 24 hours	No stain

AGA: *Amaranthus graecizans* Aerial Parts

TABLE 2
Physico-chemical Constants of the Powder of AGS

Loss of weight in drying at 105ºC	:	9.30
Absolute Alcohol solubility (%)	:	2.00
Water solubility (%)	:	20.08
Successive extractives (%)		
Petroleum ether (60-80^0)	:	1.30
Chloroform	:	1.15
Absolute alcohol	:	6.5
Ash values (%)		
Total ash	:	26.33
Water soluble ash	:	6.83
Acid insoluble ash (10% Hcl)	:	2.17
pH values (aqueous solution)		
pH of 1% solution	:	6.249
pH of 10% solution	:	5.504

*Refluxed 2.5 g of the dried powder material (AGA) with 100 ml of 10% (v/v) aqueous ethanol in a round bottom flask on a heating mantle for 4 hours. Cooled, filtered through Whatman No. 1 filter paper. Again refluxed the residue with 100 ml of fresh 10% v.v. aqueous ethanol for 4 hrs. Cooled, filtered and combined the two filtrates and removed the solvent under reduced pressure. Transferred the residue on weighed petridish and dried on waterbath till constant weight. Calculated the percentage of the extract with respect to dried powder.

Chromatographic Finger Prints of Different Extractives

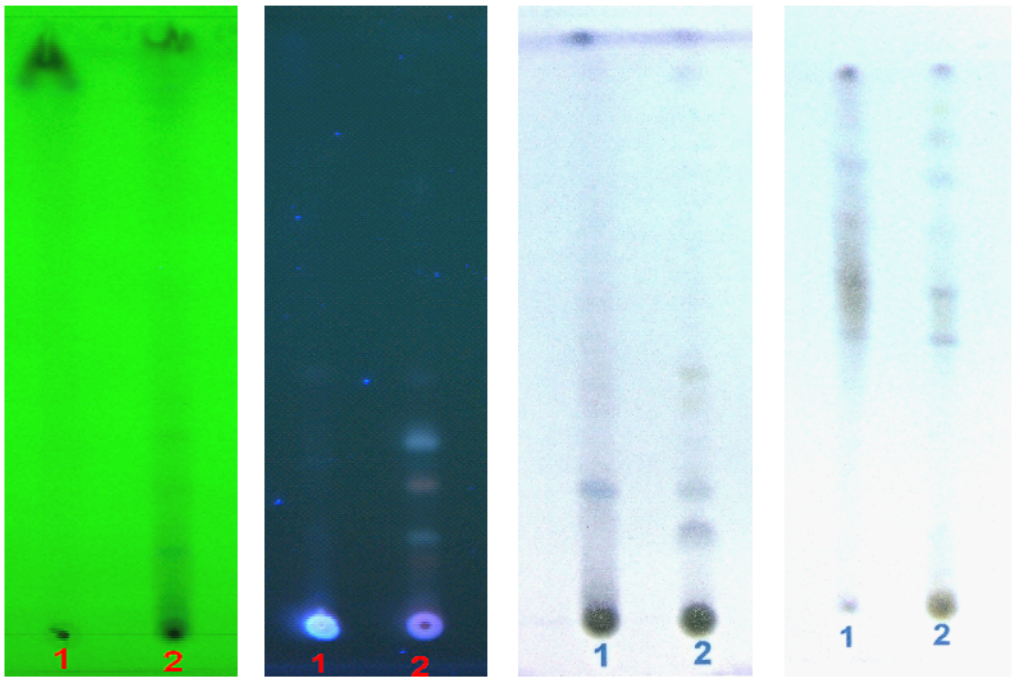


Fig. 1
Thin layer chromatographic fingerprints of AGA

Fig. A: UV254 nm, MP: Ethyl acetate-Methanol-Water (100-13.5-10)
Fig. B: UV365 nm, MP : Toluene-Ethyl acetate (93-7)
Fig. C: MP: Toluene Ethyl acetate (93:7)
Fig. D: MP: Toluene-Ethyl formate-Formic acid (5:4:1)
(**Fig. C** and **Fig. D**) After derivatization, (with Vanillin-Sulphuric acid reagent)
Track 1 Petroleum ether extract, Track 2 Methanol extract.

TABLE 3
Ultraviolet Spectrum

Apparatus used	**Milton Roy Spectronic Genesys 5 Spectrophotometer – Milton Roy**			
Sample conc. (mg/ml)	**Solvent**	**λ max (nm)**	**λ min (nm)**	**Abs. (λ max-λ min)**
1.00	Intestinal fluid simulated without pancreatic pH=7.5±0.1	No shift	No shift	—
1.23	Gastric fluid simulated without pepsin pH=1.2±0.1	No shift	No shift	—

Chemical constituents: The plant contains alkaloids, flavonoids and saponins (Ghazanfar, 1994). Aerial parts contain alkaloids, glycosides and carbohydrates, flavonoids, sterols and tannins (Abu Zaida *et al.,* 2008).

Inorganic: Results of the quantitative analysis of the ash of the AGA powder and its 10% alcoholic extractive for elements are given below in a tabular form.

TABLE 4
Ash values (*BH Pharmacopeia* Reference)

%Total Ash	**25.3136%**
%Ash insoluble in hydrochloric acid	**0.6793%**

Assay and identification of metal (AOAC international Reference)
Atomic Absorption Spectrometry

Apparatus used	**(AA-6800 Shimadzu)**				
Metal	**Std. conc. µg/ml**	**Sample conc. mg/ml**	**Flame samples absorbance**	**Flame Actual conc. mg/ml**	**Flame Actual %**
Cr	1, 2, 4	40.04	0.000	0.000	0.000
Zn	0.5, 1, 2	40.04	0.4070	0.05446	0.005446
Cu	0.5, 1, 2	40.04	0.0291	0.0098025	0.00098025
Fe	1, 2, 4	40.04	0.2914	0.3674525	0.0367452
K	1, 2, 4	40.04	2.5358	0.8106775	0.08106775
Pb	1, 2, 4	40.04	0.000	0.000	0.000
Cd	0.25, 0.5, 1	40.04	0.0017	0.0001925	0.00001925

*Total ash of AGA powder = 26-28% (with respect of dried material).

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