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PHARMACOGNOSTIC EVALUATION AND PHYTOCHEMICAL STUDIES ON THE ROOTS OF *AMARANTHUS TRICOLOR* (LINN.)

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ABSTRACT

Amaranthus tricolor Linn. belonging to the family *Amaranthaceae* is well known for curing a variety of ailments such as cough, throat infections, toothache, eczema, piles, diarrhea, gonorrhea, leucorrhoea and impotence. The present study deals with the pharmacognostic evaluation including examinations of morphological and microscopic characters, ash values, powder analysis, extractive values, moisture content and fluorescence analysis. Preliminary phytochemical screening was also carried out. Transverse section of the root showed the presence of cork cells, cortex, fibers, xylem and phloem. Total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were 12.8%, 6.89%, 5.0%, 7.6% and 20.0% w/w respectively. Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, tannins, proteins and amino acids. The study contributes to the development of standardization parameters of the plant which helps in the botanical identification of *Amaranthus tricolor* Linn.

INTRODUCTION: Herbal drugs play an important role in the healthcare programs especially in the developing countries. Ancient literature incorporates a remarkable broad definition of medicinal plants and considers all parts of the plant to be potential source of medicinal substances [1]. It is documented that 80% of the world's population has faith in the traditional medicines particularly plant drugs for their primary health care [2]. The main hindrance in the acceptance of herbal medicines is the lack of documentation and quality control. So it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine.

Amaranthus tricolor Linn. (Family- *Amaranthaceae*) commonly known as Lal Chaulai or Joseph's coat is reported to posses' good medicinal value in the traditional system of medicine. It is an annual herb of 60-120 cm in height, rooting at nodes with branching above the middle portion. The plant have branches slender not spiny often tinged with purple color, grooved, striated and glabrous. The species is found to be grown in Benin, Nigeria, Kenya, Tanzania, and Southern Africa and throughout India.

Ethno botanically, the herb is used as blood purifier, tonic in dropsy, as ascaricide, in tooth ache, sore throat, cough and bronchitis. The roots, leaves and stems are eaten in bilious disorders and are used as an aperient. Roots and seeds are used in leucorrhoea, impotence, against colic, gonorrhea, eczema and have galactagogue properties. Decoction of roots with *Cucurbita pepo* Linn. is used to control hemorrhages following abortion [3].

Roots are considered as demulcent and in the form of decoction used for piles and diarrhea in children [4]. The

plant possesses antioxidant activity, hepatoprotective activity [5], antiviral activity [6] and antiproliferative activity [7]. The plant has been screened for the presence of various phytochemicals and showed the presence of carbohydrates (free sugars, glucose and starch) [8], flavonoids (betacyanins A and B, amaranthin, isoamaranthin and quercetin) [9, 10], proteins and amino acids (proline, cysteine, tryptophan, leucine, glutamic acid, arginine, lysine, histidine, methionine, phenyl-alanine, isoleucine, tyrosine, threonine, valine) [3, 8, 9], steroids (spinasterol, cholesterol, campestrol, 24-methylene cholesterol, stigma sterol, *β*-sitosterol, fucosterol and isofucosterol) [8, 11] and fatty acids (palmitic, linoleic, lignoceric and archidic acid) [11].

As literature survey and scientific data revealed that a large number of indigenous drugs have already been investigated as regards their botany and chemistry is concerned, however a systematic standardization including Pharmacognostical and Physico-chemical study is still lacking for the roots. The present investigation of *Amaranthus tricolor* Linn. is therefore taken up to evaluate certain botanical and chemical standards which would help in crude drug identification as well as in checking adulteration, if any. Further the study will greatly help in quality assurance of finished product of herbal drugs.

MATERIALS AND METHODS:

Plant Material Collection and Authentication: Roots of *Amaranthus tricolor* Linn. were collected from the outfields of Sonepat district , Haryana. The plant was authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, NISCAIR, New-Delhi, under the voucher specimen No.- NISCAIR/RHMD/Consult/2010-11/1528/126 and a specimen was submitted to the department of Pharmacognosy and Phytochemistry, Hindu College of Pharmacy, Sonepat, Haryana (India).

Preparation of Extracts: The collected sample was washed thoroughly, dried and successively extracted with different solvents viz. petroleum ether, chloroform, ethyl acetate and ethanol so as to get the respective extracts. All the extracts were filtered individually, evaporated to dryness using the rotary evaporator, weighed and % yields were calculated as well as color and consistency of the extracts were observed.

Pharmacognostical Evaluation: Solvents viz., petroleum ether, chloroform, ethyl acetate, ethanol, n-butanol, glacial acetic acid, acetone and reagents, viz. phloroglucinol, glycerin, chloral hydrate, iodine and sodium hydroxide were procured from RFCL, Mumbai, India. Microphotographs were taken using Labomed ATC-200 microscope attached with Sony digital camera.

Macroscopic and Microscopic Evaluation: The shape, size, color, odor, taste, surface texture and fracture characteristics of the roots were determined. Transverse section (T.S.) of roots were taken using a microtome and the sections were cleared with chloral hydrate solution and stained with phloroglucinol and hydrochloric acid , then mounted in glycerin for the identification of various regions. Powder of the dried roots was separately treated with phloroglucinol, hydrochloric acid, glycerin and iodine to study various characteristics [12, 13].

Fluorescence Study: The powdered material was treated separately with different reagents and exposed to visible and ultraviolet light (U.V. short and U.V. long) to study their fluorescence behavior [14, 15].

Physicochemical Parameters and Phytochemical Evaluation: The moisture content, total ash, water soluble ash, acid insoluble ash, alcohol and water soluble extractive values were determined as a part of its physicochemical parameters [13, 16]. Petroleum ether, chloroform, ethyl acetate and ethanol extracts were subjected to phytochemical analysis for the presence of various secondary phytoconstituents using standard procedures [17, 18, 19]. Amino acids were analyzed by paper chromatography using n-butanol: glacial acetic acid: water (4:1:5) as the solvent system and ninhydrin solution (0.2%w/w) as the spray reagent [20].

RESULTS AND DISCUSSION:

Macroscopic and Microscopic Evaluation: Morphologically, the roots of *Amaranthus tricolor* Linn. appeared creamish brown in color having indistinct odor, cylindrical shape and fibrous fracture having 0.5-1 cm thickness and 10-12 length. The roots also possessed few secondary roots and numerous rootlets

with tapering end and rooting at nodules (**Fig. 1 and Fig. 2**).

FIG. 1: MORPHOLOGY OF PLANT *AMARANTHUS TRICOLOR* LINN. (WHOLE PLANT)

FIG. 2: MORPHOLOGY OF ROOTS OF *AMARANTUS TRICOLOR* LINN.

In transverse section, roots were characterized by the presence of thick brownish continuous layer of cork cells. Further, it showed the presence of cortex, phloem, xylem, vessels and fibers Fig. 3 (i) and 3 (ii). The analysis of powder showed the presence of cork cells, parenchymatous cells, vascular bundles, pitted xylem vessels (Fig. 3 (iii) 3 (iv), (v) and (vi).

FIG. 3 (I): T.S. OF ROOTS OF *AMARANTUS TRICOLOR* (LINN.)
CK=Cork cells, PT=Pith, VB=Vascular bundles, CX=Cortex

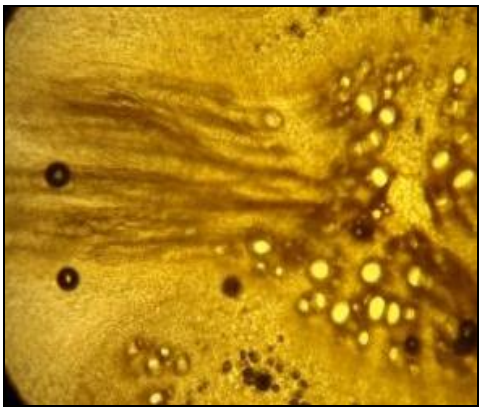

FIG. 3 (II): T.S. OF ROOT SHOWING VESSELS AND FIBRES

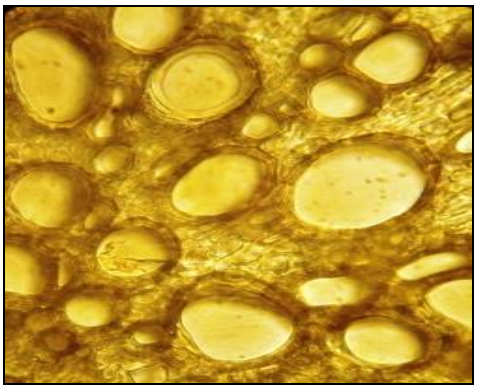

FIG. 3 (III): POWDER MICROSCOPY SHOWING XYLEM AND PHLOEM

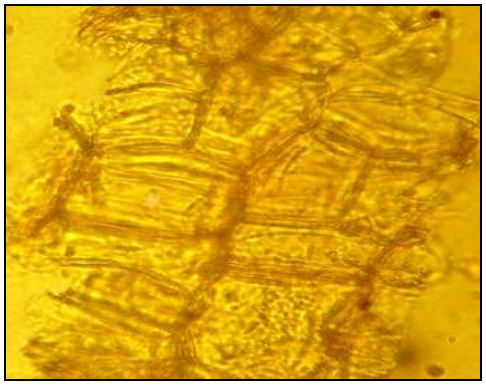

FIG. 3 (IV): POWDER MICROSCOPY SHOWING CORK CELLS

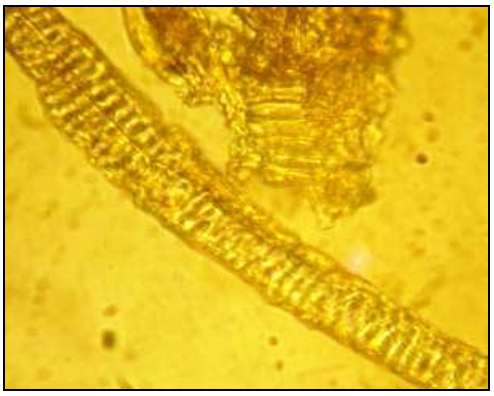

FIG. 3 (V): POWDER MICROSCOPY SHOWING PITTED VESSELS

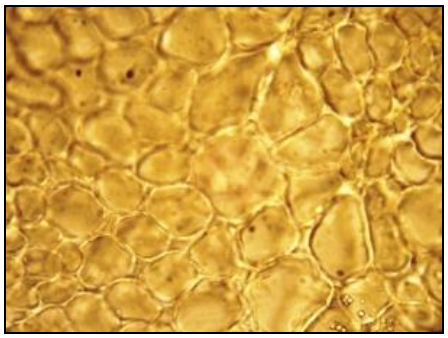

FIG. 3(VI): POWDER MICROSCOPY SHOWING PARENCHYMATOUS CELLS

Fluorescence Study: Fluorescence analysis of the various solvent extracts and powdered drug after treatment with different reagents like 1N NaOH, 1N HCl, acetic acid, picric acid, 5% ferric chloride and 5% iodine solution was observed in the day light and UV light and colors were observed. The results are shown in **Table 1** and **Table 2**.

TABLE 1: PERCENTAGE YIELD, COLOR AND THE CONSISTENCY AND FLUORESCENCE NATURE OF THE SUCCESSIVE EXTRACTS OF ROOTS OF *AMARANTHUS TRICOLOR* LINN.

Solvent used	% yield (% w/w)	Consistency of extracts	Color of extract		
			Under Visible Light	Under Short Wavelength	Under Long Wavelength
Petroleum Ether	0.45%	Sticky Semisolid	Yellowish brown	Green	Brown
Chloroform	0.96%	Solid	Reddish brown	Greenish brown	Black
Ethyl-acetate	0.64%	Semisolid	Reddish brown	Reddish brown	Brownish black
Ethanol	8.21%	Sticky Semisolid	Reddish brown	Brown	Black

TABLE 2: FLUORESCENCE ANALYSIS OF POWDER OF ROOTS OF *AMARANTHUS TRICOLOR* LINN. WITH VARIOUS CHEMICAL REAGENTS

Powder + Reagent	Visible light	U.V. Light	
		Short Wavelength	Long wavelength
Powder as such	Yellowish brown	Light brown	Brown
Powder + 1N NaOH in Methanol	Light brown	Light brown	Yellowish brown
Powder + 1N NaOH	Brown	Dark Brown	Black
Powder + 1N HCL	Brown	Yellowish brown	Dark Brown
Powder + 50% HCL	Brown	Brown	Black
Powder + 50% HNO_3	Brown	Dark Brown	Black
Powder + 50% H_2SO_4	Brown	Dark Brown	Black

Physicochemical Parameters and Phytochemical Evaluation: Physicochemical parameters are important parameters in detecting adulteration and are adopted to confirm the purity and quality of drug. Ash values are particularly important parameter as it shows the presence and absence of foreign matters like metallic salts or silica etc. Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, tannins, proteins and amino acids. Ethanolic extract also showed presence of most of phytoconstituents. The results are depicted in **Table 3** and **Table 4**. Analysis of the amino acids by paper chromatography showed the presence of *DL*-alanine and *DL-iso*-leucine.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF ROOTS OF *AMARANTHUS TRICOLOR* LINN.

Physicochemical Parameters	Results (% w/w)
Total ash	12.8
Acid-insoluble ash	6.89
Water-soluble ash	5
Loss on drying	6.7
Ethanol soluble extractive Value	7.6
Water soluble extractive Value	20
Foaming Index	More than 1000

TABLE 4: PRELIMINARY PHYTOCHEMICAL SCREENING OF ROOTS OF *AMARANTHUS TRICOLOR* LINN.

Tests for constituents	Petroleum-ether extract	Chloroform extract	Ethyl-acetate extract	Ethanol extract
Alkaloids	-	+ve	-	+ve
Carbohydrates	-	-	+ve	+ve
Flavonoids	-	+ve	+ve	+ve
Tannins and Phenolic compounds	-	-	-	+ve
Amino-acids	-	-	-	+ve
Proteins	-	-	-	+ve
Gums		-	-	-
Mucilages	-	-	-	-
Steroids	-	-	-	-
Glycosides	-	-	+ve	-
Saponins	-	-	-	+ve
Fats and fixed oils	+ve	-	-	-

CONCLUSION: It is concluded that the above parameters are very useful for the identification and authentication of the species. The results of the present study will also be helpful in preparation of monograph.

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