Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(3): 612-616

ISSN: 0975-4873

Research Article

Pharmacognostical Profiling on the Root of *Rauwolfia Serpentina*

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Available Online: 1st September 2014

ABSTRACT

Each medicinal plant and the specific plant part used as crude drug material contain active or major chemical constituents with a characteristic profile that can be used for chemical quality control and quality assurance. So the increasing demand for herbal medicines has inevitably led to maintaining the quality and purity of herbal raw materials and finished products. WHO acknowledged that Pharmacognostical standards should be proposed as a protocol for the authentication and quality assurance of herbal drugs. The important histological features of *Rauwolfia serpentina* are the cork, composed of alternating layers of broad and narrow cells giving a somewhat spongy and friable exterior to the drug; secondary cortex composed of several rows of parenchymatous cells, filled with starch grains and brown resin masses; secondary xylem consisting of vessels, tracheids, xylem parenchyma and wood fibres traversed by xylem rays, and showing annual rings; secondary phloem consisting of sieve cells, companion cells and parenchymatous cells containing starch grains, rosette and prismatic crystals of calcium oxalate and occasionally some brown resin masses.

INTRODUCTION

The increasing demand for herbal medicines, both in the developing and developed countries, has inevitably led to maintaining the quality and purity of herbal raw materials and finished products. [1] WHO, therefore, acknowledged that Pharmacognostical standards should be proposed as a protocol for the authentication and quality assurance of herbal drugs. *Rauwolfia serpentina* (Linn.) Benth. ex Kurz, belonging to the family Apocynaceae, and popularly known as India's wonder drug plant, is an upright, perennating, evergreen undershrub with tuberous roots (Fig. 1). Leaves are simple, glabrous, lanceolate or obovate and generally in whorls of three to four, crowding the upper part of the stem; flowers white or violet-tinged and borne on corymbose cymes; fruits tiny, oval, fleshy which turn shiny purple-black when ripe.[2,3,4,5] Mainly the roots of the plant are used for various ailments like insomnia, hypertension, insanity, epilepsy, intestinal disorders, cardiac and liver diseases, hysteria, constipation and schizophrenia. It is also anthelmintic, a tranquilizer and an antidote against the bites of snakes and venomous reptiles.[6,7,8,9] The characteristic fluorescence patterns emitted by the roots of *Rauvolfia* was studied under daylight and ultraviolet radiation by Selvam and Bandyopadhyay (2005).[10] The principle alkaloid of *Rauwolfia serpentina* is reserpine.[11,12] Phyto-pharmacognostical studies and quantitative determination of reserpine have been carried out in different parts of *Rauwolfia* species by Panda *et al.* (2012).[13] The present investigation was, therefore, undertaken to evaluate various qualitative and quantitative parameters on the root of *Rauwolfia serpentina*, the findings of which will be helpful in setting standards for this medicinal plant.

MATERIALS AND METHODS

The plant material, collected at an appropriate stage of its growth from NRIADD, Kolkata was authenticated through detailed taxonomic study and then air-dried for pharmacognostical study. Macroscopical study was carried out with the naked eyes/aid of a magnifying lens to determine the shape, size, texture, etc. as per requirement of Indian Herbal Pharmacopoeia. Microscopical study was performed by preparing a thin hand section of the rhizome, cleared with chloral hydrate solution and stained as per the standard protocol.[14,15,16] The dried material was coarsely powdered in a blender and subjected to various tests-powder analysis was carried out with reference to the presence or absence of particular diagnostic characters for rapid and accurate determination of their identity following the methods of Wallis (1999)[17] and Trease and Evans (2002)[16]; fluorescence analysis was carried out as per the method advocated by Chase & Pratt (1949)[18] and Harborne (1973)[19]; and physico-chemical analyses such as total ash, acid-insoluble ash, etc. on the basis of protocols prescribed by WHO on Quality Control Methods for Herbal Materials (2011)[20] and Indian Pharmacopoeia (2001)[21]. For chemical profiling of the plant, 100gm of the dried powder was subjected to cold extraction with ethanol and chloroform (1:1) for 7 days; the extract concentrated and then carried out High Performance Thin Layer Chromatography (HPTLC) following the method of Egon Stahl (2005)[22].

RESULTS

Macroscopic Analysis: Roots stout, hard, rough with irregular longitudinal fissures on the surface, sub-cylindrical to tapering, rather tortuous or curved, outer

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Fig. 1: Habit of R. serpentina

Fig. 2: Root of R. serpentina

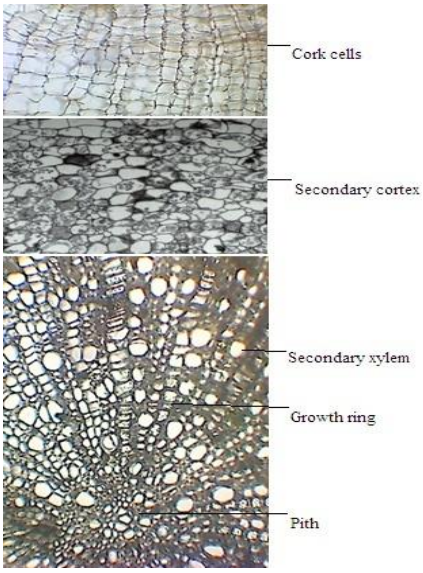

Fig. 3: T.S. of Root

Table 1: Physico-chemical Analysis

Material	Parameter	Value in % Observation I	Observation I1	Observation I11	Mean
Root powder	Foreign Matter	1.30	1.10	0.90	1.10
	Total Ash	7.20	7.40	7.20	7.27
	Acid-insoluble Ash	0.30	0.20	0.30	0.27
	Alcohol-soluble Extractive	8.10	8.00	7.80	7.97
	Water-soluble Extractive	12.90	13.50	13.10	13.17
	Moisture Content (at 105°C)	10.60	11.10	10.80	10.83

Inference (mean of triplicate): Foreign Matter=1.10%; Total Ash=7.27%; Acid-insoluble Ash=0.27%; Alcohol-soluble Extractive=7.97%; Water-soluble Extractive=13.17%; Moisture Content at 105°C=10.83%.

surface greyish-yellow to light brown and pale yellowish white inside, odour indistinct and taste bitter. Rootlets absent but few small circular root scars present. When scraped, the bark separates readily from the wood. Fracture short, irregular, the longer pieces readily breaking with a snap. The transversely cut surface shows a finely radiating xylem with clearly marked growth rings (Fig. 2).

Microscopic Analysis: Transverse section of root shows alternating strata of suberized cork cells, the strata with larger cells alternating with strata of markedly smaller cells. The secondary cortex consists of several rows of tangentially elongated to isodiametric parenchymatous cells, being filled with starch grains and brown resin masses. The secondary xylem represents the large bulk of the root and composed of vessels, tracheids, xylem parenchyma and xylem fibres traversed by xylem rays, and showing one or more annual rings with a dense core of wood at the center. Cambium is indistinct, narrow, dark and wavering. The secondary phloem is relatively narrow and consists of sieve cells, companion cells and parenchymatous cells containing rosette and prismatic crystals of calcium oxalate and occasionally some brown resin masses in outer cells and phloem rays. Starch grains mostly simple, but compound granules also occur.

Powdered-drug Analysis: Brownish to reddish grey in color, slight odour and bitter taste; characterized by spherical, simple, semi-compound and compound starch grains; rosette and prismatic crystals of calcium oxalate; brown resin masses; uniseriate medullary rays; elongated cork cells; pitted vessels with simple perforation; lignified and pitted tracheids; lignified xylem fibres, occurring singly or in small groups; parenchymatous cells containing starch grains and brown resin masses (Fig. 4).

Physico-chemical Analysis (Determination of Identity, Purity and Strength): The physico-chemical analyses of foreign matter, total ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive and moisture content taken in triplicate are shown below along with their inferences (Table 1):

Fluorescence Analysis: The powdered drug on treating with various reagents emitted the following fluorescence properties or colours under UV chamber (Table 2):

HPTLC Profile of Extract:

Sample preparation: 50 grm of dried powder of the roots of *Rauwolfia serpentina* (Sarpagandha) was subjected to cold extraction with Ethanol Chloroform (1:1) for 7 days and extract was filtered using filter paper (Whatman No 40). The whole extract was concentrated and used for the

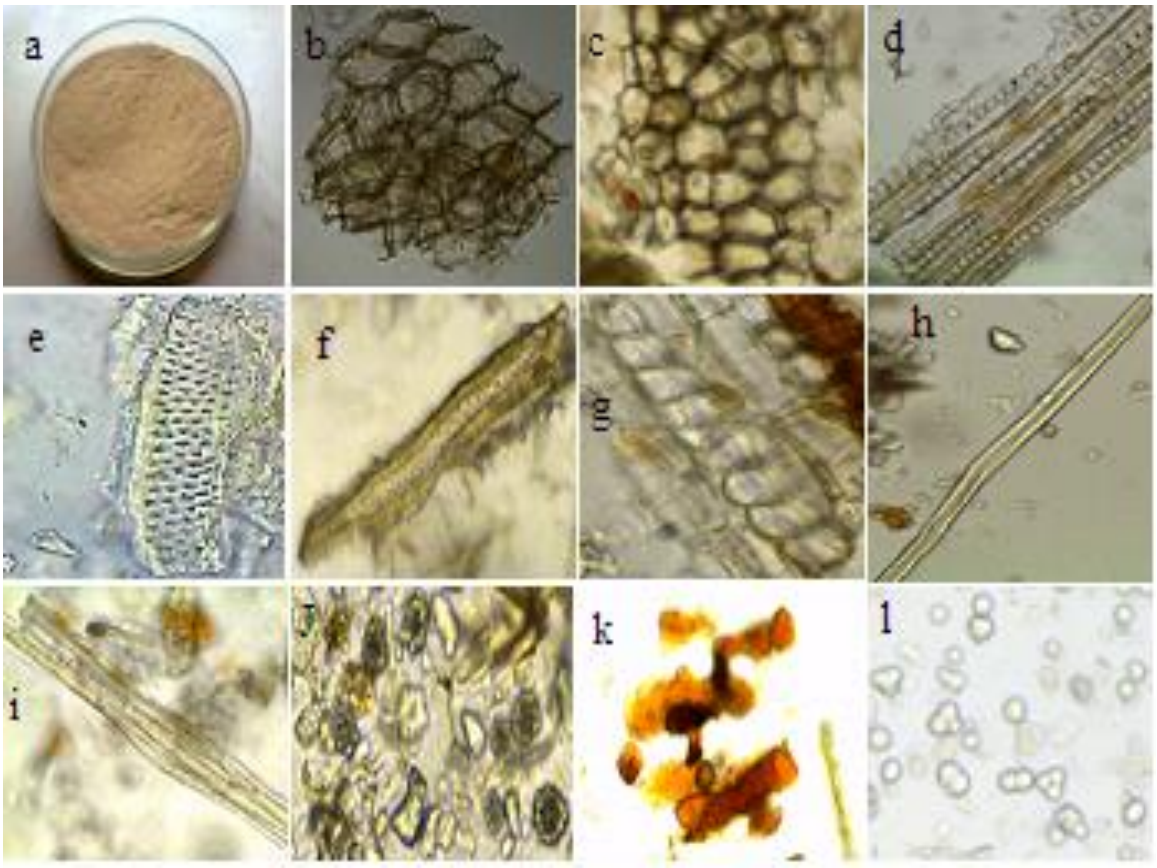

Fig. 4: Powder Microscopy - a. Powdered root; b. Elongated cork cells; c. Parenchymatous cells containing *starch grains and brown resin masses;* d. Tracheids and vessels along with medullary rays; *e. Pitted vessel with simple perforation; f. Lignified and pitted tracheid; g. Uniseriate medullary rays; h. Xylem fibre showing lumen; i. Group of fibres; j. Rosette and prismatic crystals of calcium oxalate; k. Brown resin masses; l. Simple to compound starch grains.*

Table 2: Fluorescence Analysis

Material	Solvent	Distinctive Colours Observed	
		Short UV (254nm)	Long UV (366nm)
Root powder	Water	+ ve greyish blue	+ ve whitish blue
	Methanol	+ ve sky blue	+ ve whitish blue
	Ethanol	+ ve sky blue	+ ve whitish blue
	Ethyl Acetate	+ ve faint brownish	+ ve pale greyish
	Chloroform	+ ve yellowish	+ ve creamish brown
	Pet. Ether	Dark brownish	Faint greyish

HPTLC Profile.

Stationary Phase: Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel $60F_{254}$, Mfg. by Merck, 26.09.2011, Batch No. 1.05554.0007.

Mobile Phase: Toluene: Ethyl acetate: Methanol: Formic acid (5.25:3.5: 1.25: 0.5) [GR grade solvent used , mfg. by MERCK, India].

Sample application: Applied volume 5 µL as 9 mm band and applied at 10 mm from the base of the plate. Plate size 10 X 10 cm.

Development: Developed up to 80 mm in CAMAG Twin trough chamber, plate preconditioning (temp. 40°C and relative average humidity 40%).

Derivatising Reagent**:** Dipped in 20% aqueous Sulphuric acid and charred at 105°C for 10 minutes.

DISCUSSION

Each medicinal plant and the specific plant part used as crude drug material contain active or major chemical constituents with a characteristic profile that can be used for chemical quality control and quality assurance. In the present investigation, the macroscopic study reveals the root to be hard, rough with irregular longitudinal fissures, sub-cylindrical to tapering, rarely branched, greyish-yellow to light brown outside and pale yellowish white inside, indistinct odour and bitter taste. These gross morphological characters of plant or plant part/s provide the best basis for identification, and also reveal the presence of contaminant or deterioration in a sample. The microscopical or anatomical study gives a preliminary idea about the nature and disposition of cells, tissues and cell inclusions, and thus helps understand where the

Table 3: R_f Values of the three Plates

Observed at 254nm		Observed at 366nm		Observed at white light	
R_f Values	Colour	R_f Values	Colour	R_f Values	Colour
0.09	Bluish grey	0.09	Blue	0.09	Grey
0.12	Sky blue	0.17	Faint blue	0.12	Deep grey
0.21	Light grey	0.22	Bright blue	0.17	Grey
0.36	Light grey	0.26	Sky blue	0.20	Grey
0.41	Very light grey	0.34	Sky blue	0.36	Grey
0.47	Very light grey	0.38	Light greenish	0.40	Grey
0.58	Very light grey	0.48	Light greenish	0.47	Grey
		0.76	Faint grey	0.54	Deep grey
				0.57	Grey
				0.70	Grey

Plate 1

Plate 2

Plate 3

HPTLC Fingerprints observed at 254 nm (Plate 1), 366 nm (Plate 2) and white light (Plate 3)

compounds of interest are located. Linear measurement of cells and tissues further provides better diagnostic characters for accurate identification. The internal structure of root shows alternating strata of large and small cork cells; secondary cortex composed of several rows of parenchymatous cells, filled with starch grains and brown resin masses; secondary xylem made up of vessels, tracheids, xylem parenchyma and wood fibres traversed by xylem rays, and showing annual rings; secondary phloem consisting of sieve cells, companion cells and parenchymatous cells containing starch grains, rosette and prismatic crystals of calcium oxalate and occasionally some brown resin masses. The cell contents of diagnostic value as confirmed from the powder analysis are rosette and prismatic crystals of calcium oxalate; brown resin masses; spherical, simple and compound starch grains; uniseriate medullary rays; elongated cork cells, up to 87 µm in length; pitted vessels with simple perforation, up to 345 µm in length and 58 µm in diameter; lignified and pitted tracheids; lignified xylem fibres, occurring singly or in small groups and parenchymatous cells containing starch grains and brown resin masses. Fluorescence analysis under the various reagents exhibited different shades of colour, and thus helps in fulfilling the inadequacy of physical and chemical methods for identification of the crude drug. The physico-chemical analysis is helpful in judging the identity and purity of the crude drug even from the crushed or powdered form. Every herb has a characteristic mineral content and corresponding typical ash content. So, when plant drugs are incinerated, they leave an inorganic ash which in the case of many drugs varies within fairly wide limits, and these values are of significance for the purpose of plant drug evaluation. Inorganic ash includes both 'physiological ash' derived from the plant tissue itself, and 'non- physiological ash', which is the residue of extraneous matter adhering to the plant surface. Hence, the quantitative test of total ash helps in determining both physiological and non-physiological ash. Acid-insoluble indicates the amount of silica present, especially as sand and siliceous earth; and the determination of extractive matter reveals the amount of active constituents present.

Checking moisture content helps reduce error in the estimation of actual weight of the drug material. Low moisture content is an indication of better stability against degradation of product. And determination of foreign matter enables to get the drug in pure form. HPTLC is a valuable quality assessment tool for the identification and quantification of chemical constituents present in plant drugs. The retention factor (R_f) values obtained from it can be used to identify compounds due to their uniqueness for each compound. In the present study, the R_f values of individual compounds appearing as spots vertically have been noted (the less polar compounds moving higher up the plates resulting in higher R_f values), which may thus be used as a quality control profile for this drug.

ACKNOWLEDGEMENT

We, do hereby, express our deep sense of gratitude and appreciation to the Director of NRIADD, Kolkata for permitting us to undertake this research work, thus enabling us to get the feel of doing the work and kept going till completion.

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