



E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2020; 8(6): 12-14
Received: 05-09-2020
Accepted: 09-10-2020

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Pharmacognostical standardization of *Vanasarpagandha* (*Rauvolfia tetraphylla* L.) root

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Abstract

The present communication deals on the pharmacognostical studies on the root of *vanasarpagandha* scientifically known as *Rauvolfia tetraphylla* L., which revealed some of the important diagnostic characteristics like longitudinal fissures on the outer surface of the root, greyish brown to reddish brown outer surface, compound starch grains, prismatic crystals of calcium oxalate in the cortex region, uni to biseriate medullary rays filled with starch grains, xylem vessels with pitted thickenings and elongated stone cells with broad lumen. Phytochemical studies revealed the presence of alkaloids, steroids, tannins, carbohydrates etc. Thin Layer Chromatography revealed the presence of many bio active compounds.

Keywords: *Vanasarpagandha*, *Rauvolfia tetraphylla*, *Rauvolfia serpentina*, *Sarpagandha*, Pharmacognosy

1. Introduction

The plant *Rauvolfia tetraphylla* L. (syn. *R. tomentosa* Jacq.) belongs to the family Apocynaceae is a perennial, ever green shrub reach up to 6ft. in height. The plant is native to Mexico, Central America, West Indies and northern South America and neutralized throughout the tropics including India. In most of the moist and hotter parts of India, the species is seen frequently under cultivation and often observed as an escaped plant. Leaves are whorled, elliptic; flowers greenish white or creamy white in umbellate cymes; drupes ovoid, deep purple when ripe; seeds rugose, oblong. The species is very similar to *Rauvolfia serpentina* and but the branches are harder. The roots of *R. tetraphylla* are often used as substitute or adulterant for *R. serpentina*. The plant is well known for its rich bioactive phyto-chemicals, especially alkaloids [1].

The crude extract of *R. tetraphylla* showed sedative activity [2] and significant inhibitory activity against thrombin-induced platelet aggregation [3]. The main chemical constituent's deserpine and reserpine are used in the treatment of hypertension and psychosis [4, 5, 6]. The major therapeutic claims are antihypertensive [2] and antipsychotic [7]. The major chemical constituent deserpine is used in the dosage forms like 0.25 mg/day for hypertension and 0.125 to 1 mg/day for psychosis [7].

The following chemical constituents have been reported from *R. tetraphylla* i.e. Deserpine (canescine), α -yohimbine (rauwolscine) and reserpine, ajmalicine, ajmaline, N(a)-dimethylaccedine, isoraunescine, lankanescine, pseudoreserpine, pseudoyohimbine, raunescine, sarpagine, serpine, serpentine, tetraphyllicine, tetraphylline, β -yohimbine, yohimbine, serposterol etc. [8].

1.1 Ethno-botanical uses: Roots of *R. tetraphylla* are used to stimulate uterine contraction in case of difficult delivery among the *Kaattu Naika* tribe of Wayanadu district of Kerala. Juice extracted from the root is used to treat muscular and rheumatism pain among *Paanar tribe* of Kannur district, Kerala. Root paste is taken either with milk or honey on empty stomach twice a day for 21 days to cure mental disorders by the *Malapandaram tribe* of Wayanadu district of Kerala. The root extract is given to drink 2-3 times a day for treating high blood pressure by the *Mullu Kuruma* tribe of Wayanadu district of Kerala. Root powder is used orally for 3 days by the *kuruma tribe* of Wayanadu district of Kerala for treating ulcer and as wormicidal. Root decoction and black pepper is used to expel intestinal worms in children by *Kaani tribe* of Malabar area of Kerala. About 10 ml root paste is taken orally for the treatment of snake bite by the Muthumalai forest dwellers of Tamil Nadu [9].

1.2 Regional language Names: Sanskrit: Vanya sarpagandha, Sarpanasini; Hindi: Barachandrika, chandrabhaga; Kannada: Doddachandrike, Naalkele sarpagandha; English: wild snake root, devil pepper, still tree; Malayalam: Pampumkolli, kattamalpori; Telugu: papataku; Bengali: Bar chandrika, gandhanakuli; Oriya: patalagarudi; Tamil: Pampukaalaachchedi [10].

1.3 Synonyms: *Rauvolfia canescens* L.; *Rauvolfia heterophylla* Willd. Ex Roem. & Schult.; *Rauvolfia hirsuta* Jacq.; *Rauvolfia nitida* Sesse & Moc.; *Rauvolfia odontophora* Van Heurck & Mull. Arg.; *Rauvolfia subpubescens* L.; *Rauvolfia tomentosa* Jacq [11].

2. Materials & Methods

The roots of *R. tetraphylla* were collected from a nursery at Tadasa, Shiggaon taluk of Haveri district, Karnataka State and identification confirmed by the subject experts from Survey of medicinal plant division, Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore.

The roots were shade dried, some are pulverized by mechanical grinder, passed through 40 mesh sieves and stored in a closed vessel, to carryout microscopical studies, powder studies, physico-chemical and preliminary phytochemical analysis. Macroscopical, microscopical and powder studies were carried out as per the standard procedures [12].

2.1 Hydro-Alcoholic Extract: 50 g of suitably sized powder was taken in an extractor and added 50 per cent aqueous alcohol, about 3 times the quantity of raw material and refluxed at a temperature between 80-85 °C for 3-4 hours. Filtered the extract through a Whatmann no.1 filter paper to a suitable sized vessel. The marc was extracted three times more, filtering the extract each time into the same vessel. The combined filtrates were concentrated to syrupy consistency and dried by using a rotary evaporator at a temperature not exceeding 80 °C [13].

2.2 Water Extract: 50 g of suitably sized powder was taken in an extractor and added distilled water, about 3 times the quantity of raw material and heated at a temperature between 80-85 °C for 3-4 hours. Filtered the extract through a Whatmann no.1 filter paper to a suitable sized vessel. The marc was extracted three times more, filtering the extract each time into the same vessel. The combined filtrates were concentrated to syrupy consistency and dried by using a rotary evaporator at a temperature not exceeding 80 °C [13].

2.3 Physicochemical analysis: Physico-Chemical analysis such as total ash, acid- values, extractive values, were carried out according to the standard procedures prescribed in Ayurvedic Pharmacopoeia of India and preliminary phytochemical screening of all the selected drugs were carried out as per the standard methods and procedure [14].

2.4 Preliminary phytochemical analysis: Preliminary Phytochemical screening was carried out for different extracts by using standard procedures [15, 16].

2.5 Thin Layer Chromatography (TLC): Shade dried roots coarse powder was extracted with Methanol, water-alcohol and water by reflux method. TLC studies of these extracts were carried out by using, commercially available precoated plates at room temperature

by following standard procedures [17].

3. Results

Macroscopic characteristics: Pieces of roots is about 8 to 15 cm long and 0.5 to 2 cm in thickness, sub cylindrical in shape, curved, outer surface greyish brown to reddish brown, smooth and its inner surface creamy yellow, longitudinal fissures are seen in outer surface, fracture short, odour is indistinct, taste bitter (Fig.-1&2).



Fig 1: *Rauwolfia tetraphylla*-root (tertiary roots)



Fig 2: *Rauwolfia tetraphylla*-root (mature roots)

3.1 Microscopic characteristics: T.s of the root shows outer multilayered cork, which are radially arranged, lignified, thick walled, distinct cork cambium of 1-2 layers of tangentially elongated thin walled cells, cortex parenchymatous, made up of 10 to 12 rows of thin walled parenchymatous cells, rounded, compactly arranged with little intercellular spaces containing simple rounded starch grains and calcium oxalate prisms and sclereids. Phloem consists of 10 to 12 layers of thin walled cells intercepted by single layered cambium. Xylem lignified with well-developed fibers and parenchyma, medullary rays are uniseriate to biseriate containing simple starch grains (Fig.-3 & 4).

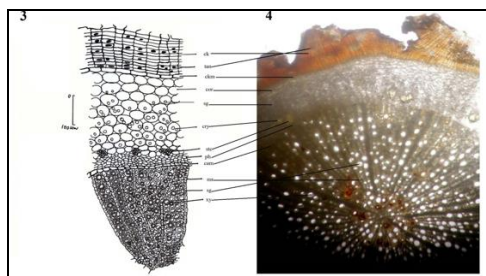


Fig 3: Transverse section of root (diagrammatic) and Fig 4: Transverse section of root (cross sectional)

3.2 Powder microscopy: Powder pale yellow shows fragments of different tissues like cork cells which are polygonal in surface view, simple to 2-3 compound starch grains with prominent hilum in the centre, thin walled tangentially elongated cork cells, xylem vessels

with prominent pits, elongated xylem fibers, thick walled elongated stone cells with broad lumen and cortex cells filled with simple and compound starch grains and prismatic crystals of calcium oxalate crystals (Fig.-5).

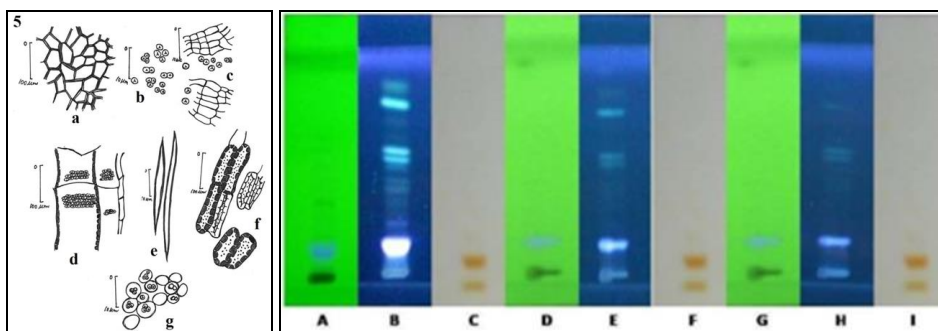


Fig 5: Various cells observed under powder microscopy and Fig 6: TLC study

3.3 Diagnostic characters

- Presence of longitudinal fissures on the outer surface of the root.
- Presence of greyish brown to reddish brown outer surface.
- Presence of compound starch grains and prism shaped crystals (Prismatic crystals of calcium oxalate) in the cortex region.
- Presence of uni to biseriate medullary rays filled with starch grains.
- Presence of pitted xylem vessels and elongated stone cells with broad lumen.

3.4 Physicochemical analysis: Physicochemical analysis has been carried out for coarse powder of *R. tetraphylla* roots, hydro-alcoholic and water extracts of root coarse powder. The powder analysis revealed loss on drying 3.36% w/w, total ash 0.80% w/w, acid-insoluble ash 0.068% w/w, pH 4.70 (10% w/v aqueous solution), water soluble extractive 8.66% w/w, Alcohol-soluble extractive 4.34% w/w and the results were showed in table1 and 2.

3.5 Preliminary Phytochemical analysis: The preliminary phytochemical analysis has been carried out for different extracts of *R. tetraphylla* – root coarse powder and the results were showed in table 3.

3.6 Thin Layer Chromatography: Thin layer chromatography was carried out on a precoated silica gel 60₂₅₄ plate by using solvent system: *Toluene: Ethyl acetate: Di ethylamine* (7:2:1). Test solution applied on a TLC plate as bands and developed the plate to a distance of 8 cm from the line of application. Dried the plate in air and examined under 254 nm & 356nm. Sprayed with *Dragendorff's reagent*. Heated the plate at 110 °C for about 5 minutes (Fig.-6).

Table 1: Physicochemical parameters *R. tetraphylla* - Root powder

Name of the parameter	Values (%) w/w
Description	Pale yellow coarse powder
pH (10 % w/v aq. solution)	4.70
Loss on drying at 105 °C	3.36
Total ash	0.80
Acid-insoluble ash	0.068
Water-soluble extractive	10.93
Alcohol-soluble extractive	4.34
Water-Alcohol extractive	11.45

Table 2: Physicochemical parameters of *R. tetraphylla* - Root extracts

Name of the parameter	Values (%) w/w	
	hydro-alcoholic extract	water extract
pH (5 % w/v aq. solution)	3.72	4.10
Loss on drying at 105 °C	4.49	5.24%
Total ash	10.10%	8.62%
Acid-insoluble ash	0.23%	0.67%
Total soluble solids	99%	95%

Table 3: Phytochemical tests for different solvent extracts of *R. tetraphylla* – Root

Natural product group	Test for natural products	Presence (+)/ Absence (-)
Alkaloids	(a) Dragendorff's test	+++ve
	(b) Hager's test	+++ve
	(c) Mayers's test	+++ve
	(d) Wagner's test	+++ve
Carbohydrates	(a) Anthrone test	+ve
	(b) Benedict's test	+ve
	(c) Fehling's test	+ve
	(d) Molisch's test	+ve
Flavonoids		-ve
Phenols	(a) Ferric chloride test	+ve
	(b) Lead acetate test	+ve
Proteins	(a) Biuret's test	+ve
	(b) Millon's test	+ve
Saponins		-ve
Starch		
Steroids	Salkowski reaction	+ve
Tannins	(a) Ferric chloride test	+ve
	(b) Lead acetate test	+ve

4. Conclusions

Pharmacognostical studies on the roots of *Vanya Sarpagandha (R. tetraphylla)* helps in the scientific evaluation, identification and authentication of drug with the help of important diagnostic characters like compound starch grains, prismatic crystals of calcium oxalate, uni to biseriate medullary rays filled with starch grains, which will be useful for the researchers and Ayurvedic physicians in differentiating with that of *sarpagandha* roots, where some times *Vanya sarpagandha* often used as substitute or adulterant for *R. serpentina*. The major difference between *R. serpentina* and *R. tetraphylla* is parenchymatous cells are pitted in *R. serpentina*, whereas pitted parenchyma is absent in *R. tetraphylla*.

5. Acknowledgement

The authors are highly thankful to the Director General, Central Council for Research in Ayurvedic Sciences (CCRAS)-New Delhi for providing the facilities to carry out the work successfully.

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