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Quality Standards, Good Agricultural and Collection Practice (GACP) of Rauvolfia serpentina (L.) Benth. Ex Kurz.

Government of Nepal
Ministry of Forests and Soil Conservation
Department of Plant Resources
Thapathali, Kathmandu, Nepal

Advisor:
Dr. Annapurna Nand Das, Director General, DPR
Mrs. Sushma Upadhyaya, Deputy Director General, DPR

Prepared by:
Rose Shrestha, Under Secretary Technical, DPR
Sangeeta Swar, Under Secretary Technical, DPR
Krishna Ram Bhattarai, Assistant Scientific Officer, DPRO, Kailali

Edited by:
Dr. Annapurna Nand Das, Director General, DPR

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Department of Plant Resources
Thapathali, Kathmandu, Nepal
Phone : 977-1-4251160
Fax : 977-1-4251141
Email : info@dpr.gov.np; department_plantresources@yahoo.com
http//www.dpr.gov.np

Photo: *Rauvolfia serpentina* Whole plant & Fresh root
(Courtesy: Rose Shrestha)

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Information Section
Department of Plant Resources
Thapathali, Kathmandu

FOREWORD

Medicinal and Aromatic Plants (MAPs) are considered as a major source of rural household income and also contribute to the local economies in Nepal. Nearly 80% of the population in the rural areas depends upon MAPs, as a source of primary health care and major income generation (WHO, 2002). Great majority of species are collected from wild and very few are practiced for cultivation. This increasing demand of the natural herbal products has opened great commercial potential. However, this has also led to over-exploitation and unsustainable collection of these resources. This has impacted negatively on their existence as well as on the environment and loss of consistency in batch-to-batch quality. Use of adulterants is another issue that further complicates the quality standards of plants-based traditional remedies.

The finished products developed using adulterared materials would become a serious health hazards. It was in this context and background and upon request of the Member States that a resolution WHA56.31 on traditional medicine was passed. Based on this, WHO developed a series of technical guidelines for ensuring quality of herbal medicine in 2003. One of them, a Good Agricultural and Collection Practices (GACP) of medicinal plants is a guidelines that provides a comprehensive framework starting from correct identification/authentication of specific protocol and package of practices for cultivation as well as pre and post-harvest handling practices for the production of MAPs and designed to ensure optimal yield of the plant for medicinal purposes in both quality and quantity without creating negative impact on sustainability as well as environment. The WHO also persuades all the countries to develop their own country specific guidelines on the basis of guidelines developed by WHO for the quality control of medicinal plants.

In this context, this booklet is prepared to provide the GACP of *Rauvolfia Serpentina* (L.)Benth. ex Kurz. (Sarpagandha). This is a fourth Nepalese country specific GACP guidelines prepared to serve stakeholders involved in promoting and commercializing this species. This report is prepared on the basis of research works carried out by the Department of Plant Resources and that from other research institutions and plant scientists in Nepal, India and abroad. Further research is required to validate the information provided in this GACP guidelines.

Dr. Annapurna Nand Das
Director General

List of Abbreviations & Acronyms

ANSAB	Asia Network for Sustainable Agriculture and Bioresources
CAMP	Conservation Assessment of Management Plan
CH_3OH	Methanol
$CHCl_3$	Chloroform (Trichloromethane)
CITES	Convention on International Trade on Endangered Species of wild fauna and flora
CSIR	Council of Science and Industrial Research
diam.	Diameter
DoF	Department of Forest
DPR	Department of Plant Resources
DPRO	District Plant Resources Office
ESON	Ethno-botanical Society of Nepal
FYM	Farm Yard Manure (Organic Compost)
GACP	Good Agricultural and Collection Practices
GoN	Government of Nepal
H_2SO_4	Sulphuric Acid
HNCC	Herbs and NTFPs Coordination Committee
HPPCL	Herb Production and Processing Company Limited
IDRC	International Development Research Center
IUCN	International Union of Conservation of Nature
MAP	Medicinal and Aromatic Plants
MAPPA	Medicinal and Aromatic Plants Program in Asia
NAOH	Sodium hydroxide
nm	Nanometer
NMPB	National Medicinal Plant Board
NTFP	Non Timber Forest Products
SDVKBS	Singhadarbar Vaidya Khana Bikas Samiti
WHA56.31	Fifty-sixth World Health Assembly on Traditional Medicine (2003) (Agenda Item 14.10)
WHO	World Health Organization

INTRODUCTION

Rauvolfia serpentina (L.) Benth Ex Kurz. (Nep. Sarpagandha, Eng. Rawolfia, Serpentina root) is an indigenous medicinal herb of Nepal having multiple influences on environment, biodiversity, rural economy, health and culture. Apart from traditional use in health care and culture, it has been increasingly used in pharmaceutical industries in the country as well as abroad. More than 30 tons of raw materials are consumed in domestic market (Tiwari *et al.* 2004). Herb Production and Processing Company Limited (HPPCL), Gorkha Ayurveda Company Limited, Dabur Nepal Pvt. Ltd. and Singha Darbar Vaidhyakhana are the major buyers in the country. America, Europe, India and Japan are the other potential market abroad. Most of its raw materials are collected from the wild while initial cultivation practices have been introduced in some parts of the country like Jhapa and Sarlahi districts. Average annual collection/ export quantity of Serpentine roots from the fiscal year 2002/2003 to 2004/2005 is 17.50 kg (DoF 2006). As per the Forest Rules and Regulations of 1995, the government has imposed a royalty of NRs. 50/kg on the collection of roots from wild (GoN 2005b).

Due to its increasing commercial demand and price in international markets, overharvesting, premature harvesting and irrational collection practices have been taking place in Nepal. It has contributed to rapid depletion of the species from its natural habitats. In Nepal *Rauvolfia serpentina* has been categorized as an endangered species, based on the IUCN Red Data Book (IUCN 2001) and critically endangered in CAMP 2001 Report (Bhattarai *et al.* 2002). It is also enlisted in CITES Appendix II. The Nepal government has banned its export in a crude form. It can be exported in processed form with the permission of the Department of Forests (GoN 2005b). It is one among 12 prioritized medicinal plant species of Nepal for research and commercial cultivation (GoN 2005a). Experimental research has been started at DPR field offices (Botanical Gardens at Banke, Brindaban and Kailali) few years back with the purpose of agro-technology development. As per research finding cultivation of *Rauvolfia serpentina* is economically viable for cultivation. The

upcoming cultivation practices in some parts of the country will further strengthen the economic viability of this species.

The quality of any raw medicinal plant and the consumer products derived from them depends on the genetic and environmental factors in addition to cultivation techniques, harvesting stages and practices, post-harvest procedures including storage and transportation etc. Inadvertent microbial or chemical contamination during any of the production stages may lead to deterioration in the quality and efficacy of the raw materials and the resultant consumer products. Therefore, it has been felt necessary that detail information on the species including its good agricultural and collection practices, post-harvest procedures and quality standards should be documented for wider dissemination and to facilitate all stakeholders in the identification, collection/production and subsequent management procedures.

1. Plant Identity

Scientific Name : *Rauvolfia serpentina* (L.) Benth. ex Kurz.

Synonym : *Ophioxylon serpentinum* L.

Family : Apocynaceae

Common Name : Serpentine, Serpentina root, Serpentine root, Rauwolfia

Local Names/Vernacular Names : Sarpagandha, Chandmaruwa, Dhaulebir (Nep); Bhujangaksi, Chandramar, Chundrika, Nakula, Nakulestha, Sarpangi. (Sans); Chyarangro (Chepang); Dharmaruwa (Darai); Baruwa, Dhaldhaliya, Pagalbuti (Tharu); God (Satar); Chandmaruwa, Chhota chand (Hindi) (ESON 2009).

Trade name : Rauwolfia, Sarpagandha

2. Parts used

Roots with bark, roots and leaves

3. Distribution

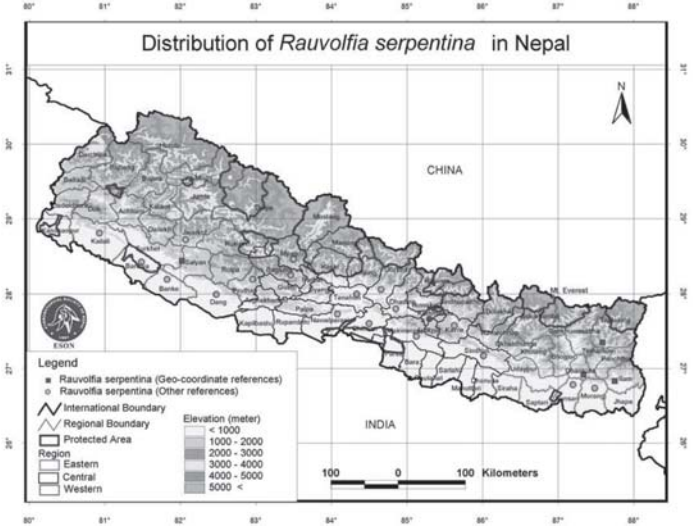
Rauwolfia is originated from South-East Asia. It is distributed in the Tropical region of Africa and America, Tropical Himalaya, India, Sri Lanka, Myanmar, Malaysia and Indonesia. In Nepal, it is found in tropical and subtropical regions from east to west along at 100-900m altitude (Hara *et al.* 1979; GoN 2007).

3.1 Ecological characters

Rauwolfia is an erect perennial under shrub, found growing in moist damp and shady places of regenerated forests. It grows more frequent under the shade of Sal (*Shorea robusta*), Harro (*Terminalia chebula*), Barro (*Terminalia bellerica*), Asna (*Terminalia alata*), Sisso (*Dalbergia sisso*), Khayar (*Acacia catechu*) and Karma (*Adina cordifolia*) (GoN 2000).

3.2 Availability in Nepal

This species has so far been reported from 17 districts (Banke, Bardia, Chitwan, Dhading, Gorkha, Ilam, Jajarkot, Kavre, Makwanpur, Morang, Myagdi, Nawalparasi, Pyuthan, Sindhuli, Sunsari, Tanahun, Taplejung) (ESON 2009)



Map 1 : Distribution of *Rauwolfia serpantina* in Nepal (ESON2009)

4. Uses

This section describes Pharmacological properties, Medicinal and Ethnomedicinal uses of *Rauvolfia serpentina.*

4.1 Pharmacological Properties

The antihypertensive effects shown by various preparations of the drug are attributed to alkaloids, especially reserpine-rescinnamine group; these effects are probably through the depletion of tissue stores of catecholamines from peripheral sites; the sedative and tranquillizing properties of the drug are thought to be related to depletion of catecholamine and serotonin from the brain (Handa *et al*. 1999; Pushpangadan 2008). A total alkaloid preparation at high concentration showed an initial stimulating action followed by paralytic action on isolated rabbit intestine, while reserpine showed paralytic action irrespective of contraction; differential activating influence of total and reserpine-free alkaloids on the activity of cathepsins indicated the extent of influence exerted by reserpine on catheptic enzyme activity (Rastogi & Mehrotra 1993). Ajmaline is found to be highly effective for the treatment of hypertension (Choudhary 2003; Joshi 2006).

4.2 Medicinal uses

- In Ayurveda, the plant root is astringent, anti-poisonous, and used in fever, worm infestation, wound and mental disorders. Root is also anthelmintic and used to cure ulcers and snake bites and scorpion sting madness (Misra 1993).

- Root bark is chiefly sedative and hypnotic and used in reducing blood pressure (Rajbhandari *et al*. 1995; Manandhar 2002). Root is considered as hypnotic, sedative, antihypertensive, specific for insanity, and its root decoction is employed in ulcers and madness (Sharma 2004) and used as anthelmintic and antidote to snakebite (Warrier *et al.* 1994). Roots constitute the drug *Rauwolfia* which has been employed for centuries for relief from nervous disorder including anxiety states, excitement,

maniacal behavior associated with psychosis, schizophrenia, insanity, insomnia and epilepsy (Ambasta *et al.*1992). Extracts of the roots are valued for intestinal troubles; aqueous decoction of root is given to cattle in diarrhoea (Dey 1998).

- Leaf juice is used for removal of opacities of the cornea of the eyes, and consumptions of fresh small pieces lower the blood pressures and facilitate baby birth and mental disorders, ulcers, snake bites and scorpion sting (GoN 2000; Baral & Kurmi 2006)

Ayurvdic preparations like Sarpagandha Vati (SDVKBS 1999), Tensarin (Gorkha Ayurved) and Reserpine (Dabur Co. Pvt. Ltd.) are available in Nepal.

4.3 Ethnomedicinal uses

- Root paste is given to cattle by the Tharu people of Dang district to treat diarrhoea (Manandhar 1985). In Chitwan district, stem is used to cure stomach pain and its root juice is drunk to cure blood pressure, kill worm and heal snake bite. Darai tribe of Chitwan district used root juice to treat stomach disorder and to get relief from malarial fever (Dangol & Gurung 1991).

- Root juice is taken by the Chepangs of Makawanpur district to treat malarial fever (Manandhar 1989; Dangol & Gurung 2000). Root extraction is given by the Limbus of Morang District to treat fever and blood pressure (Siwakoti & Siwakoti 1998).

- Root Decoction and leaves is given to cure snakebite in Satar tribe of Morang and Jhapa districts (Siwakoti & Siwakoti 2000).

5. Morphological Characteristics of plant

Rauvolfia serpentina (Sarpagandha) is a small erect or sub-erect perennial small evergreen under-shrub, 0.2-1(-2) m tall, bark pale brown, rarely lenticellate. Leaves condensed near top of stems in whorls of (2-) 3-4, rarely opposite, elliptic-lanceolate to obovate, 7-13(-17) x 2.8-6.5(-9) cm, apex acuminate to acute, base cuneate sometimes attenuate, dark green above, paler below. Inflorescence

cymose, sometimes paniculate. Flowers many, salver-shaped, white or pinkish, tube often mauve or red. Peduncles long, 4-9 cm, stout; pedicels very short. Calyx lobes triangular-lanceolate, 1-2(-3) mm long. Corolla tube slender, often curved, inflated slightly just above middle, 10-19mm; lobes obovate, 3-4 x 3 mm. Stamens subsessile, free from stigma. Ovary of 2 distinct or fused carpels, each with 2 ovules. Fruit drupaceous, black, surface smooth, solitary or more often in a conspicuously fused bifid pair. Seeds ovoid, glabrous, without wings. (Rajbhandari 2001).

6. Characteristics of Drug Materials

6.1 Diagnostic Features of Crude Drugs

Macroscopic

Roots are subcylindrical to tapering and rarely branched, rootlets usually absent. Roots are 15 to 30cm in length and 2 cm in diam.; stout, thick, tortuous. In the market roots available in 4-8cm cut pieces. Outer surface is yellowish brown with longitudinal ridges, fractures short & irregular. Internal wood is pale yellow, solid, odourless with very bitter taste (Rajbhandary *et al.* 1995; Sipahimalani, 2002; and based on field observation in Nepal)

Microscopic

Transverse section of the root presents a circular outline with typical stratified cork and other secondary features. Following are the tissues seen from the periphery to the centre. Periderm consists of stratified cork, alternating bands of smaller suberized and un-lignified cells up to 8-10 rows in radial depth and of larger, suberized but lignified cells up to 5-7 rows in radial depth; phellogen indistinct but is seen as a narrow layer of thin walled cells; phelloderm 5-7 layers, immediately below the phellem, cells are arranged in radial rows whereas away from phellem, cells are oval and have intercellular spaces. Phelloderm contains abundant starch grains (with tri-radiate hilum) and typical twin prisms of calcium oxalate. Secondary phloem is traversed by conspicuous medullary rays. Phloem consists of sieve

tubes, companion cells and phloem parenchyma. Starch grains and calcium oxalate prisms (as twins and in groups) occur throughout the phloem tissue. Secondary xylem is also traversed by well developed medullary rays. Xylem consists of vessels, wood fibres and lignified paraenchyma. The vessels appear rounded polygonal or at times radially elongated and occur either single or in pairs. Xylem fibres appear as rounded or polygonal structures with thick lignified walls. Typical oxalate prisms and starch grains resembling those of the phelloderm and phloem occur freely in the wood parenchyma. Medullary Rays run radially from the centre to the cortex through the phloem. Rays in the xylem region lignified, pitted and are 1-5 cells wide although uniseriate rays are prominent. In the phloem region the ray cells are not lignified (Sipahimalani 2002).

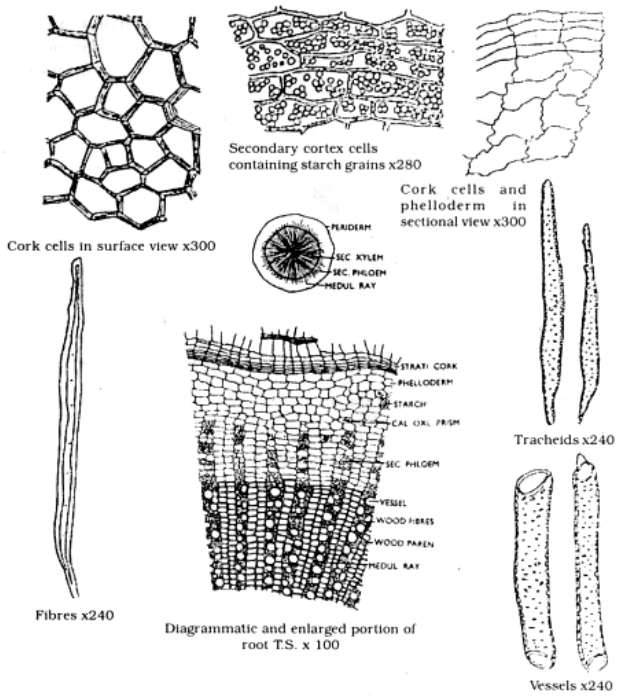


Fig. 2 : Microscopic features of Rauwolfia root (Sipahimalani 2002)

Characteristics of dry root powder

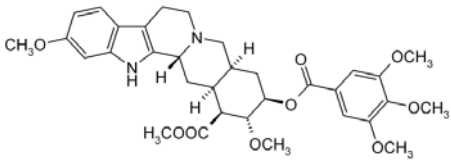
Powdered drug is yellowish brown, free flowing fine powder with strong bitter taste. It consists of fragments of cork cells. Starch grains are abundant and are mostly rounded, irregular, 9-15 μ in diam., some sowing hilum at center. Parenchyma are filled with starch grains, some are pitted with prismatic crystals of calcium oxalate; tracheids with numerous bordered pits and thickenings, xylum fibers

few with thick wall (Rajbhandary *et al.* 1995). Starch cells and typical oxalate prisms are present in the medullary ray cells. Stratified cork in several layers (some lignified) appearing like "benezene rings", in surface view is the identifying character of the powdered drug (Sipahimalani 2002).

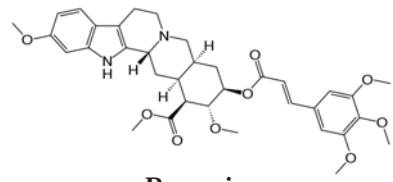
7. Major chemical constituents

The major alkaloids present in roots and root bark are reserpine, serpoterpene, ajmaline, ajmalicine, serpentine, serpentinine, serpaajmaline, yohimbine (Rastogi & Mehrotra 1993), rescinnamine, deserpidine, coryanthine, reserpiline, alstonine (Sipahimalani 2002), arginine, lycine, serine, aspartic acid, glutamic acid, thereonin, alanine, praline, valine, tyrosine, phenylanaline, iso-leucine, cystine, histadine, asparagines, glutamines, glycine, tryptophane and aminobutyric (Hussain *et al.* 1992; GoN 2007), ophioxylin, phytosterol, oleic acid (Sharma 2004), chandrine, papavarine, corynanthine, raunatine, rauvolfinine, sarpagine (Joshi 2006)

Chemical structure of major constituents



Rescinnamine



Reserpine

8. Assay/analytical methods

Determination of reserpine and rescinnamine in *Rauvolfia serpentina* preparations is done by Liquid Chromatography with florescence detection.

Prepared column - Constricted end of 30 x 2 cm id glass column is plugged with cotton. 3 g Celite 545 (Fisher Scientific Co., Pittsburgh. PA 15219) is mixed with 2 ml of 0.1N NaOH and is transferred to column. It is Compacted with rod and overlaid with pledget of cotton.

LC column – ì Porasil, 30 cm x 3.9 mm id (Water Associates, Milford. MA 01757). Any other normal phase column can be used, provided that it successfully separates reserpine from degradation products.

Reserpine stock solution - Fifty mg reserpine is transfered to 100 ml volumetric flask. 1 ml $CHCI_3$ is added, swirled to dissolve, diluted to volume with CH_3OH and mixed.

Rescinnamine stock solution – Twenty five mg rescinnamine is transfered to 100 ml volumetric flask. 0.5 ml $CHCI_3$ is added, swirled to dissolve, diluted to volume with CH_3OH and mixed.

System suitability solution - 0.50 ml reserpine stock solution is transferred to 10 ml beaker and evaporated to dryness on steam bath. 0.50 ml $CHCl_3$ is added to beaker and irradiated 10 min. under long wave UV light. $CHCI_3$ is evaporated on steam bath. Residue is dissolved in CH_3OH and solution is transferred to 50 ml volumetric flask. Beaker is rinsed with CH_3OH and rinsing are added to flask. The solution is diluted with CH_3OH and stirred.

Reserpine identification solution: 0.20 ml of reserpine stock solution is diluted to 50 ml with CH_3OH.

Reference solution for analysis of *Rauvolfia serpentina* in powdered form - 10.0 ml each of reserpine and rescinnamine stock solutions are transferred to 250 ml volumetric flask. It is diluted to volume with CH_3OH and mixed .Two 5.0 ml aliquots are transferred to separate 125 ml separating funnels, each containing 1 ml CH_3OH. Each portion is treated as follows: 60 ml 0.5N H_2SO_4 is added to separating funnel and mixed. The extract is extracted with 30 ml $CHCl_3$. $CHCl_3$ layer is transferred to prepared column and eluate is collected in 250 ml beaker. Without delay, beaker is placed on active steam bath and contents are evaporated to 25 ml with aid of strong air current, (Evaporation to 25 ml should take 30-40 min. Level of liquid should not be allowed to go much below 25 ml). 25 ml CH_3OH is added to beaker and heating is continued on steam bath until volume is again reduced to 25 ml. Solutions are transferred from both beakers to single 100 ml volumetric flask. Each beaker is rinsed

with 15 ml CH_3OH in small portions and rinsing are added to flask. The solution is diluted to the volume with CH_3OH and mixed.

Sample solution - *Rauvolfia serpentina* powder - 100 mg of powder is accurately weighed and transferred to 125 ml separating funnel containing 6 ml CH_3OH. The solution is swirled thoroughly for 1 min to disperse sample. 60 ml of 0.5N H_2SO_4 is added and mixed well. The extract is extracted with 5 successive 30 ml portions of $CHCL_3$. Each extract is added to prepared column and eluates are collected in 250 ml beaker containing 50 ml CH_3OH. The eluates are evaporated to 25 ml as described for reference solution. 25 ml CH_3OH is added to beaker and again evaporated to 25 ml. remains. The solution is transferred to 50 ml volumetric flask. Beaker is rinsed with small portions of CH_3OH and rinsing is added to flask. and then diluted to volume with CH_3OH and mixed.

Procedure for system suitability test – It is generally done to ensure the performances of analytical system so that complete testing system (including instrument, chemical reagents, column, and analysts) is suitable for the intended test. It is integral part of gas and liquid chromatography and is used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done.

It is performed only just before sample analysis. CH_3OH is eluted at the rate of 1.5 ml/min. Excitation is set at 280 nm and emission at 360 nm. Aliquot of reserpine identification solution is injected. After reserpine had eluted, flow rate is adjusted, if necessary, so that peak appears after 4-5 min. Sensitivity is adjusted so that peak height is - 70% of chart height. Aliquot of system suitability solution is injected. In addition to the reserpine peak, the chromatogram should have 2 distinct peaks, both appearing before the reserpine peak. If reserpine is not eluted after 4-5 min. or if an adequate separation of the 2 degradation products is not obtained, column performance is not satisfactory. Prolonged washing with CH_3OH may sometimes improve column performance, but if the above requirements are still not met, another column should be tried.

Determination of reserpine - Set excitation at 280 nm and emission at 360 mn. Aliquot of reference solution is injected. Sensitivity is adjusted so that peak is - 80% of chart height. Two successive aliquots of reference solution, 4 successive aliquots of sample solution, and then 2 additional aliquots of reference solution are injected respectively. If several sample solutions are to be determined in succession, this order of injection is to be followed: 2 aliquots of reference solution, 4 aliquots of sample solution I, 2 aliquots of reference solution, 4 aliquots of sample solution II, 2 aliquots of reference solution and so on.

Determination of rescinnamine - Excitation should be at 330 nm and emission at 435 nm. Process is proceeded as described for determination of reserpine (Cieri 1987; Sipahimalani 2002)

9. Quality standards

Foreign organic matter	:	Not more than 2.0%
Total ash	:	Not more than 8.0%
Acid insoluble ash	:	Not more than 2.0%
Alcohol soluble extractive	:	not less than 9%
Water soluble extractive	:	not less than 8%
Loss on drying (at 105^0C)	:	Not more than 12%

(Source: Sipahimalani 2002)

10. Collection in wild

Harvesting technique: Root is collected from the age of 2-3 years after planting. 30 months duration crop produce maximum root yield. However, for commercial purpose 15-18 months duration is feasible. Root collection is better when leaves are matured and ready to shed in early autumn season. At this stage the roots contain maximum concentration of total alkaloids. At harvest time root may be found go up to 40 cm deep in soil (NMPB 2004). Light irrigation is done for easy digging. Roots are dig up along with thin side roots, cleared and washed thoroughly and cut into 12-15cm pieces for drying and storage. Care should be taken not to keep the root bark intact (GoN 2000).

Sustainable harvesting Guidelines:

Root should be collected only from the matured plants. Tender and small roots should be left and covered by soil for regeneration. At least 20% crop should be reserved at site for sustainable production. 4-5 years rotational harvesting cycle should be adopted for sustainable production. Poor seed viability, low seed germination rate, low vegetative propagation rate, overexploitation and loss of habitat are the major causes of decline of this species from its natural habitat (GoN 2000; ANSAB 2011).

11. Preffered Growing conditions

Soil / Climate condition

Rauvolfia serpentina can grow under a wide range of climatic condition. It flourishes in a hot humid condition and can be grown both in open and partial shade.

A climate with a range of temperature from 10°C to 38°C seems to be well suited. Best areas for its growth are those which combine high rainfall (1500-4000mm) with proper drainage of the soil, such as slight slopes (Sumy *et al.* 2000).

The plant is grown in a wide variety of soils from sandy alluvial loam to red lateritic loam or stiff dark loam. Under cultivation, nitrogen may be supplied through fertilizers, farmyard manure or compost. Generally the plant produces thicker root in black stiff loamy soil or lateritic loam than in heavy clayey or sandy soil. In its natural habitat, it prefers clay or clayey loam with a large percentage of humus and then organic debris which enables uniform moisture levels and a good drainage. Slightly acidic to neutral soils with Soil pH 4-8.5 is preferable (Farooqi & Sreeramu 2001; based on field observation in Nepal).

12. Methods of cultivation

12.1 Selection of plant

Mother stock having good yield as well as high reserpine content (preferably >1.7%) and other important chemical content should be selected as the planting material.

12.2 Methods of propagation

Propagation by seeds

About 5-6 Kg seeds yields sufficient seedlings for one hectare plantation (Sharma 2004). The ripe seeds, collected from the beginning of June to the end of October or even November and stored in air tight tins, retained their viability for about six months (Farooqi & Sreeramu 2001; Field observation). Floatation test is carried out for selecting the healthy seeds. Seeds are soaked in water and preceded for germination. Generally germination percentage is found to be 20-40 %. Fresh seeds, collected from ripe fruits and immediately sown, show a higher percentage of germination (60-75%). Best time for seed sowing is in April- May. Seeds are dibbled to about 1cm deep in lines, 10-20 cm apart in raised bed. Beds are irrigated soon after sowing and kept moist by covering through husks or mulch until seed sprouts (Sumy *et al.* 2000; and based on field observation in Nepal).

Vegetative propagation

Root cuttings-Large tap roots with a few filiform lateral secondary rootlets are used. About 3-5 cm long root cutting are planted horizontally about 5 cm below the surface in nursery bed in June-July. Bed is kept moist until they sprout. In about a month, cutting starts to sprout and become ready to transplant. Root stumps about 5 cm, intact with a portion of stem above the collar can be directly transplanted in the field having irrigation facilities (Shrestha & Shrestha 2004; based on field observation in Nepal).

Stem cuttings – hard wood stem cuttings have been found better than soft wood cuttings.

Hard wooded stem cutting measuring 15 to 22 cm are planted during June in the nursery beds where continuous moisture is maintained. (GoN 2000; NMPB 2004)

12.3 Land preparation

Plantation area should be located in partially shaded areas with well drained soil and irrigation facilities. Beds are prepared in May-June with one-third quantity of well rotted FYM and two third of fine soils. Land is cleared of weeds and ploughed to a depth of 30 cm. Fifteen centimeters deep furrows are made at a distance of 45 cm for transplanting (GoN 2000; NMPB 2004).

12.4 Plantation

Seedlings of 40-50 days old having 4-6 leaves and 10-12 cm height are in right stage for plantation. The seedlings are carefully dug up with their undamaged tap-root from the nursery beds. They are immediately transplanted in the pits of 12-20 cm depth made 45cm x 30cm apart in well prepared cultivation field. (GoN 2000; NMPB 2004; based on field observation in Nepal).

13. Management

13.1 Irrigation

Transplantation is generally undertaken during the rainy season. Beds of suitable size with irrigation channels are laid. After transplantation irrigation should be given at regular intervals until the seedling get established. In hot and dry summer season irrigation should be done twice a month, otherwise monthly intervals is sufficient. Flooding is good before the harvesting of root is done (GoN 2000; NMPB 2004).

13.2 Thinning and weeding

About 2-5 weeding are done once during the monsoon and another one at the end of the growing season in December in order to maintain

satisfactory development of roots. Defloration in initial year is recommended for better root growth and yield unless seed collection is required for propagation (GoN 2000; NMPB 2004)

13.3 Manuring

The use of organic manure, leaf mould and organic compost has been recommended. A dosage of farmyard manure or compost at the rate of 30-37 cartloads (20-25 tons) per hector is applied at the time of field preparation. Mixture of manure with 20 Kg of bone meal, 2.5 Kg of ammonium sulphate and 40 Kg of leaf mould is applied during transplantation. One handful to each hole will be sufficient. (GoN 2000; NMPB 2004; Sharma 2004)

13.4 Disease and pest control

Rauvolfia serpentina is susceptible to various diseases. Viz. Leaf spots, caused by *Cercospora rauvolfiae* Chupp & Muler. and *Alternaria tenuis.* The fungus attacks on leaves also affect the flower and fruits. Mosaic is another common disease. Root knot appear as gall due to nematodes. Control by cyano gas or by flooding has been suggested. (GoN 2000; NMPB 2004)

14. Cultivation/Collection Calendar

Flowering Time :	Baisakh- Asoj (May-September)
Fruiting Time :	Asadh - Mangsir (July-November)
Harvesting Time :	Kartic - Falgun (October-February)
Seed Sowing :	Chaitra- Baisakh (April- May)
Rhizome cutting :	Asadh – Shrawan (June-July)
Plantation Time :	Jesth – Asadh (June-July)

Source: (GoN 2000; ANSAB 2011)

15. Harvesting and post harvest procedures

Harvesting and post harvest technique: Uprooting for root crop is recommended at winter (November-December). Roots of

exploitable size are collected 2-3 years after the plantation. But for commercial cultivation harvesting after 15-18 months is ideal. Before digging of roots a light irrigation is suggested. While digging of root the small fibrous branches of root should be properly handled. Collected roots are freed from the adhering soil and thoroughly washed and air dried and dried roots are stored usually in clean gunny bags. Roots should be collected with the bark intact in autumn as the bark constitutes 40-50% of the whole root and has higher alkaloid content (GoN 2000; NMPB 2004).

Productivity of roots: 0.1-4.0 Kg fresh roots are obtained from 1.5 years old plants (GoN 2007). That is the average yield of Rauwolfia dry root is approximately 2-3 ton under average management when harvested at 30 months and seedlings are raised through seeds. Plants raised from stem cutting yield about 1-2 tones/ha, and the plants from root cutting gives productivity of 3-4 tones/ha (Thapa 2001; NMPB 2004; GoN 2006).

16. Economics of cultivation

The economics of Rauvolfia cultivation has been worked out on the basis of field- research and trial (Table 1). The cost of production per hectare is NRs. 233,000 while the raw material harvested in every three years is worth NRs. 450,000. This indicates a net profit of NRs. 206,200 after three years of cultivation in one hectare of land. However apart from this general scenario there are a number of issues that may influence productivity and net income. For example the labor cost assigned for the site clearance and land preparation is applicable mostly for the first year if the proposed cultivation site is virgin. One can also reduce the cost involved in composting, plantation, and weeding, harvesting and post-harvest procedures. Likewise the cost of seedling production or purchasing them is applicable for the first year only which, in later seasons, can be substituted with the seeds and root cuttings and/or stem cuttings produced in the farm. Likewise, the selling price can be raised with the better product quality and identifying the proper market.

Table 1: Economics of *Rauvolfia serpentine* cultivation per hectare

S. No.	Description	Unit	Quantity			Rate NRs	Budget in NRs		
			1st year	2nd year	3rd year		1st year	2nd year	3rd year
1.	Seedlings	Nos.	50,000	5,000	1,000	0.5	25,000	2,500	500
2.	Compost	ton	20	7	5	1000	20,000	7,000	5000
3.	Laborer								
	Site clearing	MD	30			300	9,000		
	Land preparation	MD	60			300	18,000		
	Composting	MD	30	10	10	300	9,000	3,000	3,000
	Plantation	MD	60	10	10	300	18,000	3,000	3,000
	Irrigation	Lump sum					10,000	10,000	10,000
	Weeding	MD	40	40	40	300	12,000	12,000	12,000
	Harvesting/Post harvesting	MD			50	300			15,000
	Storage	MD			20	300			6,000
4.	Agricultural materials	Lump sum					20,000		
	Total						1,41,000	37,500	54,500
	Grand Total								2,33,000
5.	Production	Kg		3000		150			4,50,000
	Profit								2,17,000
	Contingency					5%			10,800
	Net Profit								2,06,200

Sources: Based on information generated from DPR Herbal Farms.

MD = Mandays
Kg = Kilogram

17. Market and Value Chain

Rauvolfia serpentina is one of the major traded species of Nepal occupying major share in the international trade. The ever growing demand of the raw materials from national and international markets has prompted local communities to collect more and more quantities from the wild as well as encouraging them to initiate cultivation of this species in private land, community forests and other common lands. HPPCL buys this species at the rate of NRs 100/Kg and Gorkha Ayurveda Company at 150/Kg. At International level the price of this species is US$5/Kg in Germany (ANSAB 2011).

Value chain

Value chain system is the sequence of dynamic processes from the provision of specific inputs for a particular product to primary production, transformation, marketing and distribution, and final consumption. Understanding of the position and behaviors of the stakeholders in value chain of a product is necessary to know entrepreneurship and innovation by the stakeholders in the market.

The major actors in the value chain of *Rauvolfia serpentina* are primary collectors, village-level traders, road-head traders, whole-sellers, raw material exporters, herbal enterprises, retailers, consumers and input suppliers (ANSAB 2011).

18. Adulterants/Substitutes:

The following species of *Rauvolfia serpentina* are substituted for the genuine drug.

1. *Rauvolfia. vomitoria*
2. *Rauvolfia. canescens*

While *Rauvolfia. micrantha*, *Rauvolfia. densiflora* and *Rauvolfia perakensis* are considered as adulterants.They don't contain reserpine (Sipahimalani 2002).

19. Value addition on raw materials

Value addition: Washing with uncontaminated water, complete air drying with minimum moisture content, chopping into 12-15 cm long pieces, grading, well packing in gunny bags should be done for value addition. Well packaged product should be stored in cool and aerated places.

Processing: Rauwolfia is one of the ingredients of Ayurvedic medicines used for lowering the blood pressure and mental stability. It is used in the form of Churna (Powder), Capsules and Vati (Tablets) in various Ayurvedic formulations. So processing is done for drying and making powder and capsules. Extraction of reserpine and ajmaline is also needed for drug making (ANSAB 2011).

20. Conservation Status and Measures

Rauvolfia serpentina has been categorized as endangered species, based on the IUCN threat catagories and critically endangered in CAMP workshop (Bhattarai *et al*. 2002). It is enlisted in CITES Appendix II. The Nepal Government has banned its export as a crude drug. It can be exported in processed form with permission from the Department of Forest (GoN 2005b). It is one of the prioritized medicinal plants of Nepal for research and commercial cultivation (GoN 2005a).

21. Government Royalty

According to the Forest Rules and Regulation 1995 Section 3, and its amendment in 2005, the government's royalty rate for the root is NRs. 50/Kg (GoN 2005b).

22. Authorized Institutions

The following are the authorized organizations related to the wild collection of raw materials.

1.	Collection permit	Department of Forests/Community Forest User Groups
2.	Royalty collection	Department of Forests/Community Forest User Groups
3.	Release (transit) permit	Department of Forests
4.	Local taxes	District Development Committees
5.	Certificate of origin	Federation of Nepalese Chamber of Commerce and Industries
6.	Product certification	Department of Plant Resources/ Department of Forests
7.	Export license	Department of Industries
8.	Export duty	Customs offices

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Germplasm in Vrindaban Botanical garden

Serpagandha seedlings

Roots ready to harvest

Dried snakeroots

Ripe fruits

Seeds

Rauvolfia verticellata

Rauvolfia canescens