Chapter-1
General Introduction
1.1 INTRODUCTION

Planet earth is endowed with a highly rich and diverse array of living organisms and the millions of these living organisms have been well inter-woven by the “Mother Nature”. Their genetic diversity, relationships with one another and physical environment constitutes biodiversity. The interdependence of the various life forms starting from the unicellular microorganisms to the complex higher plants and animals is a unique feature of this green planet.

Plants are one of the most fascinating creations on this earth. They capture the solar energy and transfer it to the living world. They are the source of our livelihood as they fulfill our basic needs and provide us food, cloth and shelter directly or indirectly in the form of cereals, pulses, fruits, vegetables, spices, fuel, fiber, wood, gums, resins, medicines, essential oils etc. Beside, plants have also been associated with our aesthetic, spiritual, recreational and scientific aspects and very importantly, they are also very well acclaimed as a potential source to produce a wide variety of chemical compounds of great therapeutical and economical value.

Over the centuries, people in India have a fascination and respect for the natural heritage, traditional plant ethics and tried to conserve it in varied possible ways. The sacred grooves (considering certain flora or biotic elements as divine) are a unique tradition, which has been responsible for preserving pockets of biodiversity in various parts of the country (Khan, 2001). Among the rich and varied Indian forests, the medicinal plants constitute an important part and are used for human and veterinary health care since time immemorial. Many people have defined medicinal plants in many ways, out of which the commonly accepted definition given by the Agricultural and Natural Resource Development is as follows:

“Plants that are recognized by people to have reliable and effective medicinal values, are commonly used in treating and preventing specific ailments and diseases, and play an essential role in health care.”

One important aspect of medicinal plants is the production of secondary metabolites which are being widely used by the pharmaceutical industry on a large scale. Usually these compounds are related with several important traits of the plant itself; these may be color or
fragrance of the flowers, taste and color of food, and resistance or tolerance against several pests and diseases. Besides these, they are also known to produce various compounds such as drugs, antioxidants, flavors, fragrances, dyes, insecticides, pheromones, etc.

Besides food and other basic needs, human health has gained priority in welfare programmes. Even now 80% people in the developing countries depend upon traditional medicines. Over 40% of all pharmaceuticals available in the USA depend upon natural sources. The National Cancer Institute near Washington DC has screened 29,000 plant species for potential use against cancer. About 3,000 show preliminary promise and at least five may come to rival vincristine.

Over the last decade, there has been a noticeable resurgence of interest in the use of herbal medicines all over the world including USA, Europe, Australia and Canada and several other developing countries. Although it is very difficult to calculate accurately the global market for herbal medicines, however, it is estimated at around US$ 30 billion in 2000. The worldwide medicinal plants based industry is growing at an average annual growth rate (AAGR) between 5 to 15 per cent, depending on the region. Europe leads the market, followed by Asia, North America and Japan. The USA is the fastest growing market where annual retail sale of botanical products has increased from US$ 200 million in 1988, to an estimated US$ 5.1 billion in 1997. The consumer use of these products in the USA has increased by staggering 380 percent in the past ten years. The industrial demand for medicinal plants has increased exponentially in the world market since last few decades with the emergence of newer product categories like health foods, natural cosmetics, nutraceuticals, aromaceuticals and personal hygiene products. The international trade in medicinal plants and their product was estimated at over US$ 62 billion in 2000, with average annual growth rate (AAGR) of 7 percent, and it is expected to reach US$ 5 trillion by 2050.

India occupies a prominent status across the globe, where several traditional systems of medicine such as Ayurveda, Yoga, Unani, Sidha and Homeopathy collectively called as AYUSH are being practiced since ancient time for total health care. Interestingly all these practices are mainly based on the medicinal plants. Shushruta and Charaka (1000 B.C.) recorded and compiled the properties and uses of more than 700 medicinal plants. Hippocrates (460-377 B.C.) the “Father of medicine” opined that ailments are natural. He said “Nature
cures, not the physician” and also stated “Your food shall be your medicine”. This concept evolved as a major breakthrough and herbal practices changed all over the world setting a platform for re-assessing and re-examining the plants for their health curing properties.

India is one of the 12 mega biodiversity countries of the world. According to an all India ethno-biological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India (http://www.indianmedicine.nic.in). The Glossary of Indian Medicinal Plants has listed around 3,000 plants (Asolkar et al., 1992; Chopra et al., 1956). Indian System of Medicine lists 387 plants that are used in the Ayurvedic drugs (Sarin, 1996). Similarly, the Unani system of medicine describes 440 plants (Said, 1969) out of which 360 are common to other systems generally practiced in the country. The occurrence of these medicinal plants and availability of raw materials from them is as follows:

- Plants occurring wild in forests, grasslands, aquatic and desert ecosystems, associated with other forms of natural vegetation.
- Plants growing as weed and cultivated as medicinal crops.
- Plants cultivated as ornamentals or as cereal, fruit, vegetable, spice, oil seed, essential oil or other cash crop.


In addition, almost 25% of the compounds of currently prescribed drugs are derived from plant sources (Balandrin et al., 1985). Of the estimated 25,000 flowering plant species in the world today, only about 10% have been scientifically examined for their medical applications, mostly in rudimentary way. Undoubtedly, many more plant-derived medicinal substances await discovery (Akerale et al., 1991). Most of the medicinal plants are being extracted for drug and pharmaceutical industries from wild population. This has adversely affected the very existence of a number of medicinal plants, particularly those of high commercial value. Further with the increasing demand and renewed global interest in traditional ethno pharmacy coupled with increasing performance for natural substances in the health care system, the natural wealth of medicinal plants is under tremendous pressure. Currently between 4,000 and 10,000 medicinal plants are on the endangered species list and
this number is expected to increase (Canter et al., 2005). With the changing scenario, there is a need to enhance and promote the conservation and cultivation of these natural resources especially medicinal plants. India, while following the path of development, has been sensitive to the needs of conservation. The conservation of medicinal plants has emerged as an important subject area under the **Conservation of Biological Diversity** and has been identified as one of the thrust areas under the National Action Plan on Biodiversity of Ministry of Environment and Forest. India's strategies for conservation and sustainable utilization of biodiversity in the past have comprised of providing special status and protection to biodiversity rich areas by declaring them as national parks, biosphere reserves, ecologically fragile and sensitive areas.

Despite its inherent strength in Ayurveda and other ethnic systems of medicine, India accounts for only a small portion of the world trade in medicinal and aromatic plants which is dominated by China. China holds a handsome 40 per cent share in the $62 billion world trade in medicinal plants, India accounted for a share of just US$ 100 million. The total trade in medicinal plants in India during 2004-05 was 4,530 crore, of which exports account for Rs. 3,423 crore and imports to the tune of Rs 1,107 crore. (www.pharmabiz.com/).

Global market for medicinal plants has been growing at a brisk pace of seven per cent annually, capitalizing on the growing awareness of herbal and aromatic plants worldwide. The United States accounted for nearly 50 per cent of the export of Indian medicinal plants and products. India's share in the US import of pharmaceutical preparations had steadily been increasing since 1998. One of the problems faced by the sector is destructive harvesting and inefficient, imperfect and informal marketing by pharmaceutical firms.

Out of the annual consumption of raw drugs, 50 % are from roots, 15 % fruits/seeds, 12 % wood, 9 % whole plants, 7 % bark/stem, 4 % leaves and 3 % flower (Figure-1) (timesofindia.indiatimes.com/articleshow/1026236.cms).
The National Medicinal Plants Board (NMBP), Govt. of India has identified and prioritized 32 plants for undertaking cultivation, development, formulating schemes and guidelines for financial assistance applicable both for governmental and non-governmental agencies, because of their high demand in domestic and international market.

In the last few decades, there has been a rampant increase in the interest in plant secondary metabolism. Advances in organic chemistry, extraction procedures, production technology, separation techniques, and sophistication in instrumentations has helped not only in isolating but also in elucidating structure and understanding the role of these major and minor compounds of secondary metabolism.

In biology, the concept of secondary metabolite can be attributed to Kossel (Bourgard et al., 2001). He was the first to define these metabolites as opposed to primary ones. Later on, an important step forward was made by Czapek (1921). According to him these products are derived from nitrogen metabolism by deamination and termed “secondary metabolite”. These compounds are not involved in the primary metabolic processes of the living cell, but are involved in the interaction of the organism with its environment. Each plant species has its own set of secondary metabolites, some of which may be common in many plant species or even in certain genera. About 100,000 compounds have been reported from plants, and it is estimated that every year 4000 new compounds are included in this list. After terpenoids, alkaloids comprise the second largest group of secondary metabolites, which contain many drugs and poisons. Pharmaceutical application is one of the most important uses for the pure compounds isolated from plants. These molecules can either be used directly or can be converted in to more useful products. Some compounds are produced in a small quantity in spite of having large
demand and big market, while some other compounds are produced in large quantities. This makes such compounds rather vulnerable for market fluctuations, due to several geographical, climatic and environmental, political factors. This has attracted the attention towards developing biotechnological processes for the industrial production of such chemicals.

Despite the advances made in synthetic organic chemistry, plants are still major source of a large number of prescribed medicines. This enormous versatility of plants and the biological and environmental problems associated with the conventional cultivation has provided much of the impetus behind development using plant cell and tissue culture technology over the last four decades. Being synthesized in specialized cell types and at distinct developmental stages makes their extraction, isolation and purification of important secondary metabolites difficult, thus making them high value low volume products.

Random extensive collection, overexploitation, environmental and geopolitical fluctuations further render it intricate to acquire certain plant based natural secondary products, which makes it indispensable to develop sustainable alternative source of vital natural secondary products without destroying the plant and its environment. Techniques of in vitro plant tissue culture have opened up great avenues. A well established and excellent alternative for the conservation of diversity is to resort to “test tube breeding” better known as “in vitro plant tissue culture.” (Fowler, 1980; Barz and Ellis, 1981; Shuler, 1981; Curtin, 1983; Martin, 1985; Staba, 1985).

Micropropagation, plant cell culture, transgenic microorganism, transgenic plant or plant cell cultures and isolated enzymes are certain biotechnological approaches which may be applied to produce secondary metabolites.

During the last few decades, attention towards in vitro technology, particularly the mass propagation of medicinal plants in vitro has enabled the breeders to be benefited from this technology for several reasons:

- Improved rate of plant propagation
- High throughput production of sterile plants in recalcitrant species
- Round the year availability of planting material
- Improved resistance against insect, pests, diseases and herbicides
- Production of true to type plants maintaining clonal fidelity
- Conservation of threatened and endangered plant genetic resources
In addition, transgenic research has strengthened the plant biotechnology for manipulation and exploitation of the biosynthetic capabilities of plants and plant cells. This could be achieved in the following way:

- Increased levels of secondary metabolites or molecule of interest
- Screening of novel molecules for important biological activity
- New flower color, food color
- New taste or fragrance
- Improved nutraceutical value
- Removal or lowering of toxic or anti-feedant compounds in food or fodder

Recent advances made in the field of plant cell culture and genetic manipulations have demonstrated the discovery of novel plants and genetic variants which do not exist naturally. During the past four decades significant progress has been made in the development and refinement of various tissue culture techniques to render them competence to bridge the gap between demand and supply across the globe.

1.1.1 General description of the test plant

In the 16th century, a German botanist and physician Leonard Rauwolf during his expedition to Asia had noticed this plant and described it. As an honor to him it was given the trade name Rauwolfia by Plumier in 1703. In the family Apocyanaceae Genus Rauwolfia hold an important position due to its medicinal importance along with other genera especially Catharanthus, Vinca, Tabernaemontana etc. Rauwolfia serpentina commonly known as “Sarpagandha” is an endangered plant native to India and distributed in tropical Asia and America.

1.1.1.1 Geographical distribution

Rauwolfia serpentina (L.) Benth. ex Kurz. (Syn. Ophioxylon serpentinum L.) is widely distributed in the foothills of Himalayan range, up to the elevation of 1300-1400 m and in the sub-Himalayan tract from Punjab eastwards to Nepal, Sikkim and Bhutan, in Assam, in the lower hills of Gangetic plains, Eastern and Western Ghats, in some parts of central India and in the Andamans. Beyond India, the plant is distributed in East Pakistan, Sri Lanka, Burma, Malaysia, Thailand and Java. Although the range of distribution of R. serpentina is very wide its occurrence is very sporadic. The plants usually grow scattered, very seldom close to each other. At present commercial supplies of R. serpentina roots are available mostly from the
states of Uttar Pradesh, Bihar, Orissa, West Bengal, Assam, Andhra Pradesh, Madras, Kerela, Mysore and Maharashtra. Moist forests, shady places near rain forests are the natural habitat of *R. serpentina*. The natural reserves of this plants are declining as a result of over harvesting, IUCN has kept this plant under endangered status, and it is listed in CITES Appendix II. The National Medicinal Plants Board (NMBP), Govt. of India has also placed this plant among 32 plants identified and prioritized for cultivation, development, formulating schemes and guidelines for financial assistance because of their high demand.

1.1.1.2 Related species of *R. serpentina*

The genus *Rauwolfia* comprises of around 175 species all over the world and only eight among them are indigenous to India (Sahu, 1979). These are:

1. **R. tetraphylla** L. (Syn. *R. canescens* L.; *R. heterophylla* Roem. and Schult.). In Hindi, it is named Barachandrika and is reported to be found in Bihar, Orissa, Chhattisgarh, Madhya Pradesh, West Bengal, Andhra Pradesh, Tamil Nadu, and Kerela states of India. It is native to West Indies. Its roots are often used as an adulterant of *R. serpentina*.

2. **R. densiflora** is another useful species in India which is distributed in Khasia Mountain, Deccan peninsula in the Western Ghats from Mahabaleshwar, Konkan Nilgiris, Pulneys, Annamalai hills of Tamilnadu and parts of Kerala and in Eastern Ghats region. Therapeutically this plant seems to have little value.

3. **R. decurva** Hook.f It is distributed in Deccan peninsula in the Western Ghats, Karnataka, Konkan and Poona.

4. **R. beddomei** is another large shrub, 2 m in height, is found in Western Ghats and Hilly tracts of Kerala up to 2000 feet height.

5. **R. micrantha** known as Malabar *Rauwolfia*. It occurs in Malabar. Roots have medicinal properties similar to those of *R. serpentina*.

6. **R. sumatrana** A perennial small tree, 10-12 m in height. The plants have been classified as an extra-Indian species found only in the Andaman and Nicobar biogeographic zone of Indian sub-continent. This is used extensively by the tribals of Andaman and Nicobar Islands in curing various ailments especially as an antihelminthic medicament, for relieving gastro intestinal troubles, epilepsy, fits and headache. Both leaf and bark are found to possess active ingredients of medicinal value. The species
was reported to be distributed across different islands ranging from Neil Isl. at the north to Kopenheat, Koshin and Campbell Bay, Great Nicobar at the extreme south (www.herb.com/maiti.html).

7. *R. vomitoria* Afzel and *R. caffra* both are African species. Both the species have medicinal properties similar to *R. serpentina* but with low total alkaloid content and also low in serpentine, these are currently being exploited commercially for its roots and root alkaloids in Africa.

### 1.1.2 Botanical description of the test plant

1.1.2.1 Classification

Kingdom Eukaryota

Sub-kingdom Streptophyta

Class Tracheophyta

Subclass Eudicotyledonous

Order Genitales

Family Apocyanaceae

Genus *Rauwolfia*

Species *serpentina*

#### Table-1.1: Common regional names of *R. serpentina*

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
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<tbody>
<tr>
<td>English</td>
<td><em>Rauwolfia</em> root, serpentine</td>
</tr>
<tr>
<td>Hindi</td>
<td>Candrabhaga, Chota-chand, Sarpagandha</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Sarpagandha, Chandrika, Patalguruda</td>
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<tr>
<td>Bengali</td>
<td>Chandra</td>
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<td>Assamese</td>
<td>Arachoritita</td>
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<td>Marathi</td>
<td>Harkaya, Harki</td>
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<td>Telugu</td>
<td>Patalaguni, Patalagandhi, Patalagaruda, Sarpagandha</td>
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<tr>
<td>Tamil</td>
<td>Chevanamalpodi, Sarppaganti, Sivanamalpodi</td>
</tr>
<tr>
<td>Kannada</td>
<td>Sarpangandha, Sarpagandhi, Shivanahibballi, Sutranabhi, Patalagandhi</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Amalpori, Churannavilpori, Suvapavalporiyam</td>
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<tr>
<td>Oriya</td>
<td>Patalagarur, Sanochado</td>
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<tr>
<td>Urdu</td>
<td>Asrel</td>
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<tr>
<td>Khasi</td>
<td>Todong-pait-parao</td>
</tr>
<tr>
<td>Trade Name</td>
<td>Serpentine roots</td>
</tr>
</tbody>
</table>
1.1.2.2 Morphological description

*Rauwolfia serpentina* is an erect evergreen perennial under shrub with a cluster of branches (2-6) arising from the root, attains a height up to 75 cm. to 1 m under cultivation, rootstock is long, irregularly, nodular and yellowish. Leaves are bright green simple, lanceolate, acute with short petiole in whorls of 3-5, pale yellow before shedding (Plate-1A-D). Inflorescence terminal or sometimes axillary, flowers are in compact cymes forming a hemispheric head at the end of a long peduncle. Flowers are abundant small, pedicillate, 1-2.5 cm. long, white, often tinged with violet. It flowers from March to May in Indian conditions. Fruit is drupe, single or didynamous, shining purplish black, with red pedicels. Seeds are wrinkled and ovoid. Root is prominent, tuberous, usually branched, 0.5 to 2.5 cm in diameter and grow up to 40-60 cm deep into soil. Outer bark of the root is corky with irregular longitudinal fissures and possesses high alkaloid concentration. The branches of roots contain higher alkaloid content. Fresh root emit characteristic acrid aroma and is very bitter in taste.

1.1.2.3 Cytology

Cytological studies in *R. serpentina* revealed that the chromosome number in gametic cells in this genus is \( n = 11 \) and in somatic cells it is \( 2n = 22 \). The length of chromosome varied from 1.6 to 3.4 \( \mu \). Though, tetraploids were created through colchicine treatment and mutagenesis but higher alkaloid content was not reported as compared to diploid plants (Bhaduri and Biswas, 1965; Parimoo, 1974; Mittal et al., 1979). Induced mutation in the initial stages provided up to 30% higher alkaloid yield, but in the successive generations it reverted back to its original status (Guniyal, 1988).

1.1.2.4 Cultivation

This plant is under cultivation in India, Sri Lanka, and Java. Experiments on cultivation are in progress in the United States.

1.1.2.5 Soil and Climate

It prefers clay-loam to silt-loam soils with plenty of humus and rich in nitrogenous and organic matter with good drainage. The plant requires slightly acidic to neutral soils for good growth with medium to deep well drained fertile soils. Alkaline soils are not suitable for commercial
cultivation. Generally, organic cultivation is practiced. It grows well in frost-free tropical to sub-tropical situations under irrigation. It grows luxuriantly well where the rainfall is 2500 mm or more.

1.1.2.6 Planting

It is cultivated at 45x30 cm spacing and maturity period is about 3 yrs. At this time the sub-aerial parts dry and main root reaches a depth at 0.9 meters.

1.1.2.7 Propagation

*R. serpentina* is a cross pollinated crop due to protogynous flowers. Pollen infertility varies from 91.5 % to 97.6 %. In the natural conditions it is propagated by seeds, however, under plantation conditions the crop can be propagated either by seed or vegetatively by stem cutting or root cuttings or root stumps.

1.1.2.7.1 Seed

Seed germination is highly variable. Seed viability was reported around 32 %. Germination percentage varies from 5 to 30 %. Saini and Datta (1969) examined air dried seeds of *R. serpentina* and *R. canescens*, and found out that inhibition of seed germination may be ascribed largely to cinnamic acid derivatives and to a lesser extent, to water soluble flavonoids. Germination of heavy seeds after soaking them in water for 24 hours was 20-40 % during May-June and 62.77 % germination was recorded in freshly collected heavy seeds. 6 kg of seeds is sufficient to raise one-hectare plantation. Sarpagandha takes a long duration (18-24 months) as it is a slow growing crop particularly in the initial stage.

1.1.2.7.2 Root cutting

Nearly 5 cm long root cutting are planted during spring in nursery beds. The beds are kept moist by watering. The cuttings sprout within three weeks. These can be planted in field during rainy season; the seedlings are transplanted at 45 X 30 cm distance. In this manner, an estimated 100 kg of root cuttings are found sufficient for planting one-hectare.
1.1.2.7.3  Stem cutting

Hard woody stem cutting measuring 15 to 22 cm are closely planted during June in the nursery beds where continuous moisture is maintained. After sprouting and root development, these plants are transplanted in the main field. Normally 40 to 65 percent of stem cuttings produce new sprouts (Dutta et al., 1963).

1.1.2.7.4  Root stump

About 5 cm of roots, intact with a portion of stem above the collar, are directly transplanted in the field having irrigation facilities. But this method requires a large number of plants.

1.1.2.8  Manure/Fertilizer

Farmyard manure at 20 to 25 q/ hectare is required for land preparation.

1.1.2.9  Irrigation

Sarpagandha, if grown in the areas which receive rainfall of 150 cm or above, well distributed throughout the growing season such as in Assam and Kerala, can be raised and rain fed under subtropical conditions. It needs regular irrigation with high temperature combined with low rainfall of 60-100 cm during rainy season. On an average 15 to 16 irrigations, at 20 days interval in summer season and at 30 days interval in winter season are sufficient.

1.1.2.10  Weeding

Field should be kept relatively weed-free in the early stages of growth. Two to three weedings and two hoeings in the first year where sole Sarpagandha crop is taken or 5-6 weeding where intercrops with Sarpagandha are practiced are necessary.

1.1.2.11  Harvesting/post-harvesting

Root yields at different ages and climate has shown that 18 months old crop produce maximum root yield. Transplanting is done in July; the harvesting period coincides with the shedding of leaves during early autumn in the next year. At this stage, the roots contain maximum
concentration of total alkaloids. During harvest the root length ranges between 40-90 cm and the thin roots are also collected. After digging, the roots are cleaned, washed and cut into 12 to 15 cm pieces for drying and storage. Moisture content of the dry roots ranges between 8-10 per cent. The dried roots are stored in polythene lined gunny bags in cool dry place to avoid fungal infection. The average dried root yield is 5000 to 6000 kg and average seed yield is 8 - 10 kg/ha.

1.1.3 Trade

It was recognized as a major crude drug for export long before globalization started. Major part of the commercial supply of the drug used in USA and European countries originates from India, Pakistan, Sri Lanka, Burma and Thailand. India being a major supplier holds almost a world monopoly and has been threatened with the depletion of the wild resources of the plant with the increasing national and international demand. The domestic demand for Rauwolfia serpentina roots in 2001-2002 was 424 tons, which increased to 589 tons in 2004-2005 showing an annual growth of 11.6 %. India imported 153 tons of “serpentine roots” from Myanmar during 1999-2004. Besides, India has also exported 14 tons of serpentine roots to Kuwait and United Arab Emirates in 2003-2004. Besides, serpentine roots India exported 20 tons of Rauwolfia alkaloids in tablet and other forms in 2002-2003. The major importers of Rauwolfia are USA, Germany, Italy, and Switzerland. (Source: National Medicinal Plant Board, Govt. of India). Himalaya was the first company in the world to launch, in 1934, a modern anti-hypertension drug Serpina® manufactured from Rauwolfia serpentina roots. (http://www.aboutus.org/HimalayaHealthCare.com). Since last few years its export has been banned by the Government of India. R. serpentina has been enlisted as a vulnerable species in the country many decades ago. For some years the government did not allow private leases for its exploitation, and placed it under the monopoly of a government agency TDCC (Tribal Development Cooperative Corporation). Still the commercial exploitation and supply of serpentine roots has not stopped simply because the domestic demand is still promising and ban on export has rather resulted in a decrease in its market/procurement price.
1.1.4 Uses

1.1.4.1 Pre-vedic period

There are many folk-lores about *Rauwolfia* plant. One such is that a mongoose would first chew upon its leaves to gain strength before engaging a cobra in combat. According to another, its leaves when freshly ground and applied to the toes could serve as an antidote to snake poison. A third one has it that the mentally deranged person is relieved of his madness if he eats the pieces of this root. Because of this curative effect in case of insanity, the plant has also been known in India as ‘Pagal-ki-Dawa’.

1.1.4.2 Vedic period

During this period, post vedic period gave rise to Ayurveda. In this there are references to *Rauwolfia serpentina* Benth., as Sarpagandha- snake smell or repellant, refers to the use as an antidote for snake bite. Chandra means ‘moon’ and refers to the use of plant in ‘moon disease’ or lunacy.

1.1.4.3 Medieval period

With the development of Unani and Tibbia system of medicine, the plant gained more importance. The variety of therapeutic indications attributed to the drug aroused interest among Arabian Hakims. The roots of *Rauwolfia* were used both in the form of internally and externally applied infusion or decoction in cases of snake bites and sting of insects. The plant was regarded as febrifuge, tonic, stomachic, sedative, sporofic, elampsia relief, diuretic, purgative and antihelmintic. It was also administered to promote delivery in child birth. Powder of dried roots with honey was used as a remedy in rheumatism, all poison, epilepsy, fits and eczema.

1.1.4.4 Modern period

The first important report about the therapeutic application of *R. serpentina* for hypertensive disease appeared in the work of Kirtikar and Bose (1918). Hakim Ajmal Khan, founder of the Mahatma Gandhi Ayurvedic and Unani Tibbia College, Delhi described actions of the roots of *R. serpentina* as a general sedative and cure for insanity hysteria and epilepsy (Sahu, 1979).
1.1.5 Chemistry of Rauwolfia

Dried root of *R. serpentina* is called Radix Rauwolfiae. The *Rauwolfia* species chiefly contain alkaloids, iridoids, flavonoids, terpenes, sterols, sugars and fatty acids. The total alkaloid content ranges from about 1.5-3.0 % and is concentrated in the root bark, latex vessels and secretary cells. Radix Rauwolfiae contains more than 60 indole alkaloids, the principle hypotensive alkaloids are reserpine, ajmaline, ajmalicine, serpentine and yohimbine (Figure-2). The roots of *Rauwolfia* have been found to exhibit a variety of effects such as sedation, bradycardia, myosis, ptosis, tremors, relaxation of nictating membranes and diarrhea. It is highly reputed for hypertension and is useful in stangury, fever, wounds, colic, insomnia, giddiness, anxiety states, maniacal behavior, psychosis, schizophrenia, dyspepsia, hyperglycemia and hypochondria. The roots are laxative, anthelmintic, thermogenic and diuretic. The decoction of the root is used to stimulate uterine contractions during child birth. The juice of the leaf is used for the removal of opacities of the cornea.

1.1.6 Major alkaloids of *R. serpentina*

1.1.6.1 Reserpine

Pharmacologically it is the most potent alkaloid found in *Rauwolfia*. It is a relatively weak tertiary base occurring in the oleoresin fraction of the roots. It is 3, 4 5- trimethyl benzoic and ester of reserpic acid, an indole derivative of 18- hydroxy yohimbine type. It is also present in traces in stem and leaves. It has a depressant action on central nervous system and produces sedation and lowering of blood pressure. Administration of reserpine depletes the brain and peripheral vessels of serotonin (5- hydroxy tryptamine) and catechol amines. Its primary effect on brain leads to sedation, whereas its secondary action on peripheral vessels produces antihypertensive action. Besides the amine concentration in brain it is also reported to influence the concentration of glycogen, acetylcholine, γ- amino butyric acid, nucleic acid and anti-diuretic hormones. Reserpine is now being used as a tool in physiological studies of body functions and pharmacological studies of other drugs. Reserpine is added to poultry feed for growth promotion and feed efficiency (CSIR, 1969).
1.1.6.2  Ajmaline

Ajmaline is the major alkaloid of *R. serpentina*. It is ditertiary indole base. It is pharmacologically closely related to quinidine. It has been reported to stimulate respiration and intestinal movements. It is effective against extra systoles and exhibits useful adjunctive action in auricular fibrillation and a few other heart conditions. It is used in arrhythmia as it slows down the rhythm due to potent sodium channel blocking properties (Rolf *et al.*, 2003). Ajmaline produces no sedation. Ajmaline may be useful in combination with antihypertensive agents for the treatment of hypertension complicated by a cardiac condition.

1.1.6.3  Ajmalicine (Raubascine)

It is a stereo isomer of tetrahydro alstonine. It possesses a central depressant activity in addition to its adrenergic blocking activity. Ajmalicine causes hypotension with renal vasodilation. It is sympatholytic.

1.1.6.4  Serpentine

It is a yellow quaternary indolic anhydronium base. Serpentine causes marked inhibition of succinate dehydrogenase in brain and liver tissues. It produces systemic and pulmonary hypotension due to a decrease in cardiac output. It inhibits intestinal movements.

1.1.6.5  Yohimbine

Yohimbine (rauwolsicine) causes hypotension. It is reported to be cardiovascular depressant with hypnotic activity. It is a 2-adrenoceptor antagonist with potential clinical applications in erectile dysfunction.
Figure-2: Important alkaloids of *R. serpentina*; (1) reserpine; (2) ajmaline; (3) ajmalicine; (4) serpentine and (5) yohimbine
1.1.7 Aims and objectives of the study

The increasing demand of *Rauwolfia* in national and international markets and decreasing availability have encouraged many innovative herb growers of to cultivate this useful herb, but the farmers are facing many problems. The main problem is the duration of crop which requires 18-24 months. In these regions, the cost of planting material is very high and the wild habitat forests are the only source for procuring herb. As high cost of cultivation is involved, the cost of propagules (roots of cultivated origin) automatically increases. This is really surprising and depressing that national buyers prefer the roots of bigger size which have only traces of alkaloids. The *Rauwolfia* roots from wild collected are not true to the species and is stated to be a mixture of *R. serpentina* and *R. tetraphylla* or other related species. In order to root out the problem of poor germination, the basic scientific studies are essential. The problem of poor germination has forced the farmers to use root cuttings for propagation. The increasing demand of root cuttings is again becoming a problem for natural population. So, keeping in view the above problems and overwhelming interest in the techniques of plant tissue culture, the present study was aimed at the following objectives:

1. Production of quality planting material: Mass propagation of *Rauwolfia serpentina* plantlets employing bioreactors.
2. Enhancement of *Agrobacterium rhizogenes* mediated hairy root biomass and secondary metabolite(s) yields of selected reserpine yielding hairy root clone of *R. serpentina* through media optimization (chemical and physical parameters) and addition of elicitors.
3. Generation of variability in the biochemical / chemical profiles of hairy root clones through induction, establishment and critical evaluation of several independently raised root clones.
4. Molecular analysis of the *in vitro* raised somaclones.

Keeping in view the above objectives whole course of the present study has been covered in two separate chapters. Each of the chapters comprises of relevant introduction, detailed review of literature, material and methods and results followed by thorough discussion.