GENERAL INTRODUCTION

Plants have for centuries been an important source of precursors and products used in a variety of industries, including those of pharmaceuticals, food. cosmetics and agrochemicals. Use of plants as drugs goes back to prehistoric times. The old Egyptians, Babylonians, Greeks, Roman, Chinese and Indians, all developed their characteristic materia medica. It is a remarkable circumstance that, alongside of modern medicine, the mediaeval Graeco-Arab system known as 'Unani Tibbi' (Ionian Medicine) and the ancient healing system of Ayurveda continue to hold the field in the Indian subcontinent, catering to the needs of a large majority of the population.

In the 1920's a concerted effort was made at the School of Tropical Medicine in Calcutta, towards the pharmacological investigation of the active constituents and isolation of a whole series of plant drugs. Subsequently, as a more purposeful approach from the point of view of the indigenous systems of medicine, a drug research institute was established at the Ayurvedic and Unani Tibbi College in Delhi, on the initiative of late physician and statesman Hakim Ajmal Khan. The very first drug that came up for investigation at this institute was *Rauvolfia serpentina*, which was systematically used over a long period by the late Hakim Ajmal Khan in the treatment of a variety of mental ailments.

The extraordinary medicinal properties of *R. serpentina* have brought this Indian drug plant to the attention of the medical world. The alkaloids obtained from its roots have the property of lowering blood pressure and are, consequently, widely used for a long time in the treatment of hypertension. With the help of clinical trial Rath *et al.* (1999) have observed similar effect of ayurvedic preparation containing *R. serpentina* to that of Atenonol 50. The drug is widely used as a sedative, hypotensive, tranquillizer in insanity and a host of other mental

ailments (Akram *et al.*, 1993; Roja and Heble, 1996). Its antirhythmic property was observed by Vollosovich *et al.* (1978). Very recently Pravathi Devi (2000) mentioned that the plant can be used to prevent menopausal problems and helps to keep the woman as feminine forever.

The *Rauvolfia* genus of Apocynaceae consists of more than 130 species (Woodson *et al.*, 1957), of which *R. serpentina* was the most important one. The name was assigned by the French botanist Plumeir in honour of the 16th century German physician and botanist Leonhart Rauwolf, who travelled extensively in the Middle East searching for plants with medicinal usefulness. Later the name of the genus *Rauwolfia* has been changed to *Rauvolfia* on the basis of a rule of Botanical Nomenclature.

R. serpentina is a small erect woody shrub, usually less than a meter in height and which bears attractive white or pink flowers. It flourishes in the moist deciduous forests of India, usually between sea level and 120 m. It grows wild along the Western and Eastern Ghats, in the lower hills of the Gangetic plain, and on the lower Himalayan slopes from Simba to Assam (Santapau, 1956; Bal, 1956; Kaul, 1956). The root system consists of permanent tuberous soft tap root up to 6 cm in diameter when fresh and having a corky bark with longitudinal fissures. In North Bengal once the plant was mentioned by J. D. Hooker as dominant species in Terai and Dooars region but from survey of the plant appears to be an endangered species as not a single plant of this species is available in wild condition (Nair *et al.*, 1991).

In spite of the wide reputation of *Rauvolfia* extracts in folk medicine, the curiosity of chemists was not attracted to their investigation until 1931. Greshoff in 1890 noted the presence of alkaloids in *R. canescens* L., but no further work was reported until 1931 when from the roots of *R. serpentina*, obtained from the Bihar province of India, Siddiqui and Siddiqui isolated a series of crystalline bases, namely, ajmaline, ajmalinine, ajmalicine, serpentine and serpentinine.

taking advantage of the differences in their basic strength on the one hand, and the solubility of their hydrochlorides on the other. Later in 1939, working on the roots and root bark of the plant, obtained from the more temperate climate of the Dun Valley, Siddiqui reported the isolation of two isomers of ajmaline, namely isoajmaline and neoajmaline, a base melting at 220°C and from the neutral fraction of the alcoholic extract, a white crystalline alkaloid melting at 234°C, with the yield of 0.1% on dry weight basis. From this last mention base and its mother liquors it was subsequently possible through repeated fractional crystallisations from methanol and acetone to isolate a crystalline substance, which melted at 270–273°C, and yielded on mild hydrolysis an acid and a base. These findings, however, were not reported, awaiting further work on the problem which had to be held over during the war and its aftermath.

In 1952, Swiss chemists Muller *et al.* reported the isolation of this base under the name, reserpine, from the so-called "oleoresin" fraction which actually corresponds to the 'neutral fraction' referred to above. Reserpine soon proved to have a very important therapeutic value and during the ensuing five years an intensive search was made for alternate sources of this alkaloid. By the end of 1956, the constituents of more than 20 *Rauwolfia* spp have been examined, most of which were shown to contain reserpine. In addition, a total of approximately 40 well authenticated alkaloids had been isolated, together with several less known ones, which have not yet been fully characterized.

It was not until 1815 that the term pharmacognosy was introduced by C. A. Seydler, a medical student in Halle, Germany. The name is termed out of two Greek words "pharmacon", the drug and "gnosis"—knowledge. The most comprehensive idea of the scope of pharmacognosy was presented by Fluckiger—who stated that it is the simultaneous application of various scientific disciplenes with the objective of acquiring knowledge of drugs from every point of view (Tyler *et al.*, 1976). In a restricted sense the definition of pharmacognosy implies

the study of plants yielding natural product having bioactivity from botanical and chemical point of view.

The cultivation of *R. serpentina*, which remains the best source of reserpine, has now become of highest importance, since the unrestricted harvesting of the plants soon threatened to exhaust the supplies available in India. This led the Indian government to encourage and foster its cultivation on a large scale.

In conventional method, the propagation of *R. serpentina* is done by seeds, root cuttings, stem cuttings and root stumps. It is usually propagated by seeds. The percentage of germination of seed is reported to be 10 to 50% (Badhwar *et al.*, 1956a; Hedayatullah, 1959; Nayar, 1956; Santapau, 1956). Due to poor and erratic germination of seeds and as such the collection of seeds from wild sources is both laborious and costly, vegetative propagation has been advocated for raising plantations. Badhwar *et al.* (1956b) compared the different methods of propagation of *R. serpentina* and their effect on root production.

A few reports indicate that some growth physiological and agronomical studies have been carried out with *R. serpentina*. Nandi and Chatterjee (1975) studied the effect of NPK fertilizers singly or in combination on growth and alkaloid formation in *R. serpentina* at high altitude of Darjeeling Himalayas. Their results revealed that high level of P only augmented the laminar area, whereas N when applied both singly or in combination, decreased it. Single application of N and K could not increase the total alkaloid contents. Maximum alkaloid synthesis was noticed in combination of N, P and K. Sahu (1970 a, b) and Maheswari *et al.* (1988) also studied the effect of fertilizers on growth, root and alkaloid yield of *R. serpentina* and reported similar observations. Maurya *et al.* (1999) observed that 60 kg N/ha was suitable for higher root yield of *R. serpentina* in Bihar, India.

Less information is available on irrigation scheduling of *R. serpentina*. Maheswari *et al.* (1991) observed that dry root and alkaloid yields and water use efficiency of *R. serpentina* increased up to an IW:CPE of 0.75. The alkaloid content was not affected by irrigation schedules.

Removal of flowering branches and cutting of flower buds have been recorded in the literature as the means of increasing the root yield along with the intensive formation of the secondary plant products. Biswas (1969, 70, 71, 73) studied the changes in the alkaloid contents of *R. serpentina* under defloration and nitrogen treatments. Complete defloration resulted in large increase of root weight. An appreciable increase in the total alkaloidal contents of the moisture free root was also observed.

Saini and Mukherjee (1970) also observed increased root yield due to complete defloration, but the alkaloid content per unit weight of dry root did not change with complete defloration.

Mass scale of collection of *R. serpentina* from natural habitats is leading to a depletion of plant resources. Therefore, efforts towards systematic cultivation, propagation, conservation and genetic upgrading for productivity and quality of existing genetic stocks are important (Mathur *et al.*, 1993). Barnah and Nath (2000) observed ecophysiological adaptability of *R. serpentina*.

Propagation of *R. serpentina* by means of seed is unsatisfactory, due to unfavourable climatic conditions. like varying temperature and humidity prevailing during the months of July and August which cause shrivelling and desiccation of the endosperm. This hinders the development of embryos and causes the formation of non-viable seeds of large number. The germination percentage of viable seeds is very low in the plant grown in plains of Darjeeling district. Thus there is enough scope to study germination behaviour of seed of the plant for their large scale propagation.

Other means of propagation are by root stumps and by root, stem and leaf cuttings. Of these the only method which gives a high percentage of successful plants is the propagation by root cuttings (Sitaram, 1978). However, the alkaloidal content of roots from plants raised by root cutting is less than those raised from seeds. Furthermore, the roots are not cherished for propagation by the growers due to their demand in the drug market

Biotechnology has slowly evolved during the early fifties and may be said to have come of age in agriculture only in the eighties. The pioneering researches include the discovery of phytochrome and its role in switching plant advanches permits splicing and annealing DNA sequences at all. Now a days the definitions are usually oriented towards processes and industry related products. Thus in 1981 European Federation of Biotechnology defined it as "integrated use of biochemistry, microbiology and chemical engineering in order to achieve the technological application in the capacities of microbes and cultured tissues. In 1982 organization of the Economic Corporation and Development defined biotechnology as the "application of scientific and engineering principle to the processing of materials by biological agents to provide good services".

Dr. T. B. Kenorcy, Jr. Administrator, Agricultural Research Service (USDA) (1986) defined it as "those biological means used to develop process and products employing organisms or their components" (Dasgupta, 1991).

The world population is nearing 5 billions and this rate of growth it is likely to touch 7.5 billions within a few years (Rajendra and D'Souza, 1999). The primary health care of the people is a necessity and the nature can only provide the needed resources. The *in vitro* culture of ayurvedic medicinal plants can be used for the supply of plants to the people and ayurvedic practitioners. The *in vitro* culture of plants with a view to getting secondary metabolites is not encouraging. Comparison made between whole plants and cell cultures show that using conventional whole plants is more practical and economical (Chem and Zu,

1996). Recently a large number of scientists are, therefore, engaged in micropropagation and *in vitro* preservation of plants known to have therapeutical properties.

Commercialization of tissue culture of medicinal plants, however, has received a poor response compared to that of ornamental plants. It is estimated that worldwide approximately only 5% of the total production of medicinal plants is through tissue culture. However, in India and other developing countries commercial production of medicinal plants through tissue culture is only 0.1% of the total production, though with lower wages plants are being produced at much cheaper rates. Though some medicinal plants are being produced commercially in India through tissue culture, but some important plants specially *Rauvolfia serpentina* are needed large quantities as they are in great demand (Rajendra and D'Souza, 1999), special attention and efforts have to be made for commercial exploitation of these plants.

There were previous attempts for the propagation of *R. serpentina* through tissue culture (Akram and Ilahi, 1986; Ilahi and Akram, 1987; Ilahi *et al.*, 1988; Akram *et al.*, 1993; Ruyter *et al.*, 1991; Roy *et al.*, 1995; Sarker *et al.*, 1996; Roja and Heble, 1996). The roots of the regenerated plants were compared with conventionally cultivated *Rauvolfia* plants for the presence of indole alkaloids (Ruyter, 1991). Vomelenine has been observed to be produced during cell culture of *R. serpentina* (Stockigt *et al.*, 1981; Chand *et al.*, 1999). Recently Akhtar *et al.* (2001) reviewed root culture of various medicinal plants with special emphasis on productivity of secondary metabolites though Benjamine *et al.* (1994) reported the production of reserpine during hairy root culture of *R. serpentina.* The hydroquinone; o-glucosyl transferase has been isolated from cultivated *Rauvolfia* cells (Arned *et al.*, 2000).

With this background investigation has been carried out on *R. serpentina* under various treatments and conditions in the ecological condition of Darjeeling district of West Bengal with the following objectives:

(i) Extraction and purification of reserpine available in *R. serpentina* and to develop an easy colorimetric method for its quantitative estimation.

(ii) To study the different methods of propagation of *R. serpentina.*

(iii) To study the growth and root and alkaloid yields of *R. serpentina* in relation to NPK fertilizers and irrigation.

(iv) To establish an efficient protocol for micropropagation of *R. serpentina* using different growth regulators. An attempt to isolate and estimate alkaloids from *in vitro* and *in vivo* grown plant materials was also undertaken.