Chapter 2

REVIEW OF LITERATURE

Valerinaceae is a well-known medicinal family, the members of which have long been used as sedatives in Europe and Asia. The important species of this family are *V. officinalis*, *V. jatamansi*, *N. jatamansi* and *V. glechomifolia*. In addition, all the plant species of this family grow in a wide variety of habitats which include shady, moist, slopy, and rocky areas.

2.1 General work on valerian

In northern Europe, the official drug in the British and European Pharmacopeias is derived from *V. officinalis* L., but other species are also used as crude drugs in other parts of the world, the most notable being Indian valerian, *Valeriana jatamansi*, Chinese and Japanese valerian *V. angustifolia* Tausch. and *V. fauriei* Briq. (Mathela, 2009).

The dried rhizome and roots of *V. officinalis* are used medicinally in certain cardiac ailments. The fresh juice of the rhizomes and roots containing a volatile oil is used against nervous disorders and certain cardiac disease. Root oil is also used in perfumery and tobacco flavoring industry for musky, woody and balsamic notes. The volatile oil produced from genus Valeriana consists of a mixture of mono and sesquiterpenes. The latter class of compounds is of great interest both from the chemotaxonomy point of view as well as from their biological activity (Bos et al., 1997). The therapeutic properties of valerian are attributed to a group of compounds known as valepotriates. The valepotriates are a group of monoterpenoids of iriddoid type having epoxy group and beta-acetoxy isovaleric acids. The roots/rhizomes are highly aromatic and contain valepotriates and essential oils, both perhaps contributing towards its
medicinal properties. In addition, sesquiterpenoids, monoterpenoids, lignans and alkaloids with pharmacological properties have been reported in *V. jatamansi* (Tang *et al.*, 2002; Gupta *et al.*, 2004; Bos *et al.*, 1997; Kumar *et al.*, 2008; Mathela and Dev, 2003; Kulkarni *et al.*, 1964; Mathela *et al.*, 2005; Sati *et al.*, 2005; Mathela *et al.*, 2006; Mathela *et al.*, 2007).

A system for growing in liquid medium whole plants of *V. glechomifolia*, endemic to southern Brazil and capable of accumulating bioactive valepotriates, was studied by Russowski *et al.* (2006). Murashige and Skoog (MS) and Gamborg (B5) media without phytohormones were evaluated after four weeks of culture in relation to growth and valepotriate yield. Valtrate was the most abundant valepotriate, followed by acevaltrate and didrovaltrate.

Violon *et al.* (1984) reported *in vitro* callus and root differentiated tissue cultures of *V. officinalis*, grown in continuous dark condition showed in several cases higher valepotriate contents than the roots of intact plants.

Propagated valerian has been shown to maintain stable valepotriate production comparable to or even higher than field-grown plants. Micropropagated *V. officinalis* plants showed less variation than seed propagated plants in contents of active constituents (Gao and Bjork, 2000). In another study performed with *V. edulis*, Castillo *et al.* (2002) showed that the contents of valepotriates from roots and rhizomes of *in vitro* regenerated plants were comparable to the same parts of wild plants in reproductive stage. Micropropagated plants of *V. glechomifolia* showed similar or higher contents of valepotriates compared to wild plants after three months of acclimatization (De Carvalho *et al.*, 2004).
Singh et al. (2006) developed a simple, rapid, cost-effective and accurate high performance thin layer chromatographic method for quantification of valerenic acid, one of the key marker compounds in case of *V. jatamansi* and *V. officinalis*.

### 2.2 Medicinal properties of *V. jatamansi*

*V. jatamansi* has been used in Ayurvedic, Unani and modern system of medicine due to its high medicinal and aromatic values. The species has been claimed to possess sedative, neuroprotective, anxiolytic, anticonvulsant and antistress activities (Bhattacharyya *et al.*, 2007; Rehni *et al.*, 2007, Wasowski *et al.*, 2002) for which used in several Ayurvedic preparations (i.e., Sudarshan Churna, Pipalayasava, Dasan galep, etc.), and known to cure obesity, skin diseases, insanity, epilepsy and snake poisoning (Prakash, 1999). Sedative, antispasmodic and tranquilizing properties of the plant are due to the presence of essential oil and nonglycosidic iridoid esters known as valepotriates (Gupta *et al.*, 1986). Essential oil from rhizomes exhibited antifungal and antibacterial activities (Girgune *et al.*, 1980). 6-methylapigenin and hesperidin isolated from rhizome of Indian valerian showed anxiolytic and sedative activities (Wasowski *et al.*, 2002). The major valepotriates are valtrate, acevaltrate and didrovaltrate due to which *V. jatamansi* possesses antispasmodic, anticonvulsive and antidepressant properties (Gupta *et al.*, 1986). Didrovaltrate, valtrate and their degraded product baldrinal were found to be cytotoxic in rat hepatoma cells (Bounthanh *et al.*, 1981). In addition, the antitumor activity of didrovaltrate was demonstrated in vivo on female mice KREBS II ascetic tumors (Marder *et al.*, 2003). Also, the group reported that 6-methylapigenin and hesperidin, isolates of *V. jatamansi* rhizome extract are the members of natural flavonoids showed tranquilizing activity on the central nervous system (CNS). Sah *et al.*
(2011) reported antidepressant like effect of essential oil of *V. jatamansi* in both acute and chronic treatment.

### 2.3 Essential oil production of *V. jatamansi*

Bos *et al.* (1997) analysed the essential oils isolated from rhizomes of *V. jatamansi* from commercially available plant material of various origins. The essential oil content of *V. jatamansi* varied between 0.09% and 1.30%. The main components were \( \gamma \)-patchoulen, \( \alpha \)-humulene, \( \alpha \)-bulnesene, bornyl isovalerate and patchouli alcohol. Also, the group mentioned that the composition of essential oil isolated from roots and rhizomes of *V. jatamansi* varied considerably depending on the origin of the plant material. However, patchouli alcohol, and \( \beta \)- and \( \gamma \)-patchoulen seem to be characteristic for this oil, as these compounds have not been described to be present in other Valeriana species.

Extensive characterization of essential oil of *V. jatamansi* from Western Himalayas was carried out (Mathela *et al.*, 2005, Sati and Mathela, 2005, Gupta *et al.*, 2004, Mathela *et al.*, 2009), which described the presence of three distinct chemotypes (maaliol and patchouli alcohol and kanokonyl acetate) of the species. A total of 100 samples of *V. jatamansi* were collected from Nainital, Bageshwar, and Almora districts in Uttarakhal (India) and analysed by GC and GC-MS. The result revealed that maaliol, patchouli alcohol and 8-acetoxypatchouli alcohol were the major constituents present in the essential oil (Mathela *et al.*, 2005).

For the first time Mathela *et al.* (2009) reported kanokonyl acetate as a major constituent found in volatile oil of Indian valerian. In the same report, sesquiterpenes were shown to be the main constituents (89.3%) comprising kanokonyl acetate (42.4%), \( \gamma \)-curcumene (10.7%), \( ar \)-curcumene (7.2%), \( \beta \)-farnesene (3.2%), xanthorrhizol (4.1%),
7-epi-α-selinene (2.2%), valeranone (2.0%) and curcuphenol (1.4%). Furthermore, these three chemotypes grow in separate areas and were not noticed as a mixed population in the natural habitat (Mathela et al., 2009). Most reports were related to plant materials collected from Kumaon and Garhwal regions of Western Himalayas.

2.4 Tissue culture of V. jatamansi

Very limited work has been done on tissue culture of V. jatamansi with few fragmentary reports on in vitro propagation and plantlet regeneration (Kaur et al., 1999; Mathur and Ahuja, 1991). Mathur and Ahuja (1991) reported the effect of NAA (3 mg/l) and Kn (0.25 mg/l) on callus induction using petiole explants. But the result was not satisfactory with respect to standardize the protocol for shoot regeneration from callus. MS medium supplemented with 3% sucrose and BA (1.0 mg l⁻¹) was used for multiple shoot induction in which 3–6 shoots were developed from single in vitro axillary bud (Kaur et al., 1999).

Hairy roots of V. jatamansi were obtained following co-cultivation of detached leaf explants with Agrobacterium rhizogenes strains. The culture medium did not show the presence of any valepotriates or their decomposition products, suggesting that the retention of total valepotriates within the tissue mass (Banerjee et al., 1998).

2.5 Characterization: Phenotypic, genotypic and biochemical

Valeriana jatamansi is displaying variability in various phenotypic traits at various stages in its populations. This wide distribution in trait values is the raw material for operative evolutionary forces and natural selection. This phenotypic variability speaks the language of a typical meiotic and breeding system, which generate the variability in Valeriana jatamansi (Aabid et al., 2012). The result revealed that in response to their highly specific ecological environments this species have developed a spectacular
diversity in their morphological characters viz., plant height, leaf number and dimensions, number of ramets, floral density, root length and number, rhizome dimensions etc. Detailed morphological studies not only give specific botanical identity to a species but such studies reveal interesting features which are helpful in understanding the range of morphological variations present across different ecological zones.

However, it was also observed that plants growing under shade respond to shade by allocating more biomass to leaves and hence registers maximum leaf number and dimensions as well as plant height to compete for light, as holds true of many other plant species. In natural environment plants can adapt to extreme differences in irradiance at several integration levels. Plants grown under shade have lower net photosynthetic rate and leaf mass per leaf area unit and these two parameters affect the productivity of a plant. *V. jatamansi* as an endangered and threatened species of India (Kaul and Handa, 2000) thrives well under shaded and moist conditions. Large scale production of this plant under artificial shade is economically not feasible. Therefore Vats et al. (2002) studied the photosynthetic response to irradiance in *V. jatamansi* and was able to develop a protocol to grow these plants under high photosynthetic photon flux density (PPFD) and therefore this Himalayan species may be cultivated in open habitat to meet the ever increasing industrial demand. Maximum amount of biomass is allocated to the organs of support i.e., rhizome and stem followed by leaves and inflorescences which is more in plants grown in shady habitat as holds true of other plants growing in shady environments of the forests (Abrahmson and Gadgil, 1973; Abrahmson, 1979).

*V. jatamansi* is a natural tetraploid species indigenous to the Indian Himalaya. Rajkumar et al. (2011) assessed its genetic diversity and population structure. Six natural populations from the western Himalayan region were analyzed using amplified fragment length polymorphism (AFLP). An analysis of molecular variance found that 93% of the
genetic variation of *V. jatamansi* was within populations and 7% among populations. In the present study, high genetic diversity was observed at the species level (*P* = 71.37; *I* = 0.409), which is higher than the other species of Valeriana. *V. walrothii*, a natural tetraploid species found in Italy, showed less species diversity than *V. jatamansi* (Grassi *et al.*, 2004). Similarly, the genetic heterozygosity of *V. jatamansi* populations was higher than that of another endemic species, *V. ciliate* (Faivre and Windus 2002). Compared with those endemic species, *V. jatamansi* is widely distributed, which explains its higher genetic diversity.

Apart from essential oil, the therapeutic properties of valerian are attributed to a group of compounds known as valepotriates which are a group of monoterpenoids of irridoid type having epoxy group and beta-acetoxy isovaleric acids. Valepotriates were characterized by HPLC and confirmed by NMR (Tang *et al.*, 2002; Gupta *et al.*, 2004). Previous studies on *V. jatamansi* revealed the presence of fifteen iridoids, eleven flavones and three sesquiterpenoids (Singh *et al.*, 2006). The chromatographic separation of the acetone fraction from the roots of *V. jatamansi* led to the isolation of 11-methoxyviburtinal, baldrinal, prinsepiol-4-O-β-D-glucoside, coniferin, and hexacosanic acid. A simple, rapid, cost-effective and accurate high performance thin layer chromatographic method has been developed for quantification of valerenic acid in *V. jatamansi* (Singh *et al.*, 2006). Separation and quantification was achieved by HPTLC using ternary mobile phase of hexane: ethyl acetate: acetic acid (80:20:0.5 v/v).

### 2.6 Conservation of medicinal plants

Continuous loss of habitat due to deforestation and industrialization is a major threat to many medicinal and aromatic species in both developing and developed countries (Shanley and Luz, 2003; Schippmann *et al.*, 2006). In tropical areas changing
Moreover, a system of preliminary screening of micropropagated plants to identify the variants could be considered as important.

2.7 Role of Vesicular arbuscular mycorrhizae (VAM) in micropropagation system

*In vitro* mycorrhization (Pons *et al.*, 1983) refers to the inoculation of AMF to the roots of micropropagated plantlets growing on agar medium. Although, the technique of *in vitro* mycorrhization is of utmost importance for the development of micropropagated plantlets, contamination of the inoculum, behavior of the host *in vitro*, and obligate nature of the endophyte are some major hurdles in the establishment of mycorrhizal host symbiosis *in vitro* (Schubert *et al.*, 1990; Rai, 2001). To circumvent this problem, an alternative method, *ex vitro* inoculation or *in vivo* mycorrhization can be used, which is relatively easy, natural, feasible and easily implementable method and shows no technical problems associated with *in vitro* mycorrhization. In *ex vitro* inoculation, the AMF spores are inoculated to the micropropagated plantlets after transplanting into pots (Rai, 2001).

It has been reported that biopriming of micropropagated plantlets with arbuscular mycorrhizal fungi (AMF) improves plant performance and plays a significant role in ensuring the health of plantlets (Rai, 2001). So, the judicious use of AMF can provide an alternative and effective approach for ensuring the proper establishment of *in vitro* propagated plantlets under field conditions.

During acclimatization, the plantlets must increase absorption of water and minerals as well as the photosynthetic rate (Grattapaglia and Machado, 1990). AMF are well known to increase the vigor of plants by increasing absorption of water and mineral nutrients, especially phosphorus. Moreover, AMF can protect host plants from root pathogens and mitigate the effects of extreme variations in temperature, pH and water...
stress (Siqueira, 1994). Successful AMF inoculation at the beginning of acclimatization period (Estrada-Luna and Davies, 2003) or even during in vitro conditions (Mathur and Vyas, 1995) has been demonstrated.

Biopriming of micropropagated plantlets with AMF helps in the development of a superior and stronger root system (Ponton et al., 1990) by increasing the rooting intensity and surface area of existing roots (Puthur et al., 1998). Colonization of a plant root by AMF can alter the morphology of a root system in a structural, spatial, quantitative and temporal manner (Norman et al., 1996). The AMF colonized roots are highly branched, i.e., the root system contain shorter, more branched, adventitious roots of larger diameters and lower specific root lengths (Atkinson et al., 1994). As a direct consequence, mycorrhizal inoculation stimulates rooting and growth and thereby transplant survival of cuttings and seedlings raised in the nutrient media.

AMF associations have been shown to improve photosynthetic efficiency by improving P nutrition in plants (Marschner, 1995), owing to an effect of P status on CO2 assimilatory reactions. The rate of photosynthesis, for instance, is strongly influenced by inorganic P concentration in the chloroplast (Herold, 1980), and P deficiency in plants leads to an inhibition of photosynthesis (Jacob and Lawlor, 1991). It has been shown that chlorophyll concentration in AMF treated plants is higher than their nonmycorrhizal counterparts (Kapoor and Bhatnagar, 2007). Mycorrhizal structures effectively take up P from lower concentrations in the soil at which normal plant roots fail (Jeffries et al., 2003). AMF helps in increasing nutrient uptake by increasing the surface area of absorptive system (roots) of plants and exploring soil by extraradical hyphae beyond the root hair and P-depletion zone. The absorbed P is then converted to polyphosphate granules in external hyphae and passed to the arbuscule for transfer to the host plant.
(Azcon-Aguilar and Barea, 1996). A similar mechanism is employed for the uptake of K, Zn, Fe, Cu, Mg and Ca.

AMF have been well documented as biocontrol agents, and the general conclusion is that they can reduce or even suppress, damage caused by soil borne pathogens. AMF colonized plants have been shown to display a significant degree of bioprotection against various pathogens (Slezack et al., 2000; Elsen et al., 2001) like Fusarium (cause wilt), Phytophthora, Aphanomyces, Verticillium (Azcon-Aguilar and Barea, 1996) and Nematodes causing respectively root rots, lesions, wilts and galls (Pinochet et al., 1996).