7.0 Summary and Conclusion

*Picrorhiza kurrooa* Royle is a medicinal herb belonging to family scrophulariaceae (Bentham, 1835). The species is found in higher altitudes on Himalayas at an elevation of more than 8000 m above mean sea level (Pannell, 1943). In western Himalayas of Kashmir the species is reported to be found near Aphanwat Kashmir (Rau, 1975); Brazil pass (Pannell, 1943); Sonsa Nag 10000-13000ft (Coventry, 1927); Pir Panchal pass (Pannell, 1943); Krishan Ganga valley (Kapahi et al., 1993); Sonamarg (Pannell, 1943). *Picrorhiza kurrooa* propagates vegetatively, such species are at high risk due to non viability of the plant propagules/seeds. *Picrorhiza kurrooa* grows in colonies and are not found distributed over large distances. The genus *Picrorhiza* is included in Appendix II of CITES (Convention on International Trade in Endangered Species), hence *Picrorhiza kurrooa* is considered as endangered in the IUCN (International Union for Conservation of Nature and Natural Resources) Red Data Book (Mulliken, 2000). Over 17,000 plant species in India, 7000 species are considered to have medicinal uses (Groombridge, 1992). For the sustainable use of such resources conservation of genetic diversity is essential to maintain ecological and socio economic equilibrium. The objective of conservation is to maintain genetic identity as well as integrity of the species in its natural habitat. Hence documentation of genetic variation is of vital significance to evolve conservation and artificial propagation strategies with long term impact. Genetic variation can be directly assessed through genetically controlled molecular markers. These markers involve assessment of variation directly at DNA level or through phenotypic expression that can be protein or chemotypic variants. Various molecular approaches viz; Random Amplified Polymorphic DNA (RAPD) assay and PCR-RFLP of ITS were used to evaluate the genetic differences or similarities between 60 accessions of *Picrorhiza kurrooa* isolated from three ecogeographical locations. RAPD was found
efficient marker in differentiating *Picrorhiza kurrooa* populations. Of 15 random primers used, only 3 generated the polymorphism. The percentage of polymorphic loci ($P$), Shannon information index ($I$) and Nei’s gene diversity index ($h$) equaled 40%, 0.254 and 0.177 respectively as obtained by an online statistical analysis tool (Popgene version 1.31). Genetic variation among the populations was higher than that within populations, and the genetic variability occurred mainly among the populations. According to Nei’s $G_{ST}$ (0.95) calculated (Popgene version 1.31), the gene differentiation among the populations was conspicuous, indicating low gene flow among the populations $N_m$ (0.026). In contrast PCR-RFLP showed bias in detecting exact species variation. Of the 19 restriction enzymes used for restriction digestion no marker revealed variation in the ITS1-5.8S-ITS2 region of ribosomal DNA. To authenticate the results obtained by two markers, sequence analysis of ITS region of ribosomal DNA (ITS1 and ITS2, including 5.8S rDNA) was performed. Three independent accessions of each population from Sonamarg, Gurez and Matayin were sequenced. Phylogenetic relationship inferred from ITS sequences is in agreement with RAPD data. For further species discrimination microsatellite DNA markers were developed from *Picrorhiza kurrooa*. A higher diversity in microsatellite length polymorphism at a locus containing a repeat sequence was observed (Fig 24). After getting the exact inter and intraspecific variations by the use of molecular markers, the chemoprofiling of the accessions was performed by two bioactive markers picroside I and picroside II. A LCMS method was developed to separate and quantify the marker compounds. The studies have revealed that sample specimen of *Picrorhiza kurrooa* from Matayin (Ladakh) contained higher concentration of picroside II (8.895%) were as picroside I was found in higher quantity (8.370%) in a specimen collected from Gurez (Kashmir). This work will be highly useful to control the quality and quantity of *Picrorhiza kurrooa* during its preparation and application in the clinic.