Chapter 7

SUMMARY

The historic use of medicinal plants from high elevations in healthcare systems emphasises the value of preserving biodiversity and opens up new research possibilities. The present study aims to investigate the genetic and molecular differences among medicinal plants used in Uttarakhand, India for various ailments. Conservation of plant resources, preservation, and systematic cultivation of medicinal plants outside their natural habitat have become a priority in the last two decades due to their depletion caused by various reasons. India, a tropical country, is home to many medicinal plant species, but like other developing nations, the biodiversity of medicinal plants is threatened by their overexploitation by pharmaceutical companies.

Rheum species are herbaceous plants with fleshy roots that grow perennially. They grow from short, broad rhizomes with a deciduous core and have upright stems with basal leaves that are deciduous. Many species of *Rheum* have been used as nutraceuticals for almost 2,000 years in Asia. Oxalic acid is commonly found in all plant parts but its concentration is quite low in the parts used for food preparation, such as leaf stems and petioles. The tart flavour of the plant is due to the nontoxic component, malic acid. Apart from oxalic acid, other harmful substances produced by *Rheum* plants include citric acid and glycosides which are present in leaf blades. If taken in excessive quantities, leaf blades, whether raw or cooked, are dangerous to both animals and people. *Rheum* species can be propagated by seeds or by cutting up the crowns of large plants.

Understanding the genetic differences in terms of quantity, quality, and arrangement is crucial in genetics research. This knowledge serves as the foundation for evolutionary testing and is also essential for conducting phytochemical analyses, in vitro cultivation of germ-plasm, and developing strategies for plant conservation and preservation. Creating effective methods for examining genetic diversity can offer fresh perspectives into how populations are sustained and evolve over time. The identification and examination of genetic variations can assist in understanding the molecular basis of different biological processes in plants. Molecular markers and their association with observable traits can serve as valuable reference points for understanding genetic variation. There are various molecular marker techniques that can be used to estimate the

genetic and molecular diversity in different geographical areas of a population. Examples of these techniques include Inter-Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR) & Randomly Amplified Polymorphic DNA (RAPD) etc. RAPD technology is a method that utilizes short primers with random nucleic sequences to amplify unidentified DNA sequences, enabling the detection of significant levels of DNA variation. ISSR, a recently developed molecular technique, analyses differences in microsatellite regions spread across plant genomes and characterizes individual gene loci more effectively than other molecular approaches. It is commonly used for evaluating the plant diversity and plant population genetics and structures. DNA-based molecular marker analysis helps geneticists to monitor the precise distinction in gene sequences in different genotypes at different times, which is important for plant breeders to evaluate the genetic combinations of characters selected for breeding strategies over the generations. This information provides insight into the genetic belonging to a specific taxon for future selection based on DNA markers.

In the present research work nine populations of *Rheum* species were collected from different altitude from the Rudraprayag district of Uttarakhand. The prime objectives of the present work were designed to evaluate the genetic diversity among the different populations so as to yield the valuable information regarding the diversity and variability within and among the populations/provenances of plants which otherwise would take several years to yield this information through conventional methods. In addition to this the impact of microbial and chemical elicitors on the composition and quantity of the biologically active component is also analysed by estimating the phytochemicals present in the samples and determining their antimicrobial activity. For genetic diversity analysis RAPD, ISSR markers were used.

The genetic diversity analysis using molecular markers, DNA should be of high quality and purity, and free from contaminants such as proteins, RNA, and other organic molecules that may interfere with molecular marker analysis. In the current investigation, the DNA was isolated from 9 different landraces of varying altitude using a bit modification in CTAB method. Every population comprises of 12 samples, so total of 108 samples were evaluated for genetic diversity analysis. The quality of DNA was evaluated using spectrophotometry and gel electrophoresis, and stored for preservation at -20°C till further use for RAPD and ISSR analysis. For RAPD analysis 15 decamer primers were selected on the basis of literature, out of them 12 primers resulted in distinct

and reproducible bands. The study involved analysing 108 plant samples of *Rheum emodi* using 12 RAPD markers. A total of 142 fragments were observed, with an average of 11.8 fragments per primer. Among these fragments, 65.64% exhibited polymorphism. This indicates a substantial level of genetic variability within the populations of *Rheum emodi*. An average polymorphic content (PIC) 0.39 was evaluated. On the basis of AMOVA analysis percentage variation among population was only 5.04 % and within population 94.96 %. This could be one of the reasons for declining diversity of *Rheum* species. The AMOVA (F_{ST}) was found to be 0.05 indicating low levels of genetic differentiation among populations. The RAPD marker indicated that *Rheum* species populations are genetically highly diverse. Similarly, ISSR markers also showed the high level of polymorphism that is 62.84 %. The mean value of PIC observed was 0.40. The AMOVA analysis unveiled substantial genetic variations among the populations under investigation, surpassing the variations within each population for both markers. More precisely, 2.7% of the total molecular variance was attributed to diversity among populations, while the majority (97.30%) of the variance was attributed to variations within each population. The AMOVA (F_{ST}) was found to be 0.27 indicating low levels of genetic differentiation among populations and higher value 1.16 of gene flow for ISSR markers.

The use of different markers for the population of *Rheum emodi* showed significant genetic diversity among the population collected from different altitude. The findings were very well supported by AMOVA and dendogram analysis. The dendogram showed the division of populations on the basis of altitude, divided into three ranges: lower, middle and higher. It was clear from the dendogram that most of the populations were placed according to the altitudinal range.

In addition, a screening was conducted on seven populations of *Rheum* species that were located at different altitudes. The objective of this screening was to identify the presence of various phytochemicals, and it involved the use of four different solvents. For preparation of extracts, the powdered leaf samples were extracted with Chloroform, methanol, acetone and water using a soxhlet apparatus. The extract so obtained was dried and further dissolved in mother solvent for further use. The phytochemical screening of *Rheum* species samples revealed the presence of various compounds such as amino acids, alkaloids, flavonoids, steroids, fats and oils, tannins and phenols, carbohydrates, and glycosides in different samples. Additionally, a thin-layer

chromatography (TLC) method was developed to quantify emodin simultaneously in the leaf extracts of *Rheum emodi*. The combination of Hexane, Chloroform, and acetic acid in a ratio of 6:1:0.1 (v/v/v) was used as the solvent system, which resulted in effective separation of emodin without any interference from other compounds present in the extracts. To ensure optimal resolution and consistent retention factors (Rf), the TLC chamber was saturated with the mobile phase for 30 minutes. The Rf value of emodin was determined to be 0.6 cm±0.03 and 0.5 cm±0.03 cm, respectively, for standard concentrations of 0.2 mg/mL and 0.5 mg/mL. The Chloroform variants of Pothivasa, Tungnath, Chopta and the Acetone extract of Dugalbitta showed the same retention factor values as of standard thereby confirming the presence of emodin in the given samples. The concentration of emodin was estimated using standard curve calibration that came out to be 0.227 mg/mL in the Chloroform variant of Tungnath. The concentration is very low in comparison with the literature studies. The observed differences and variations in the content of compounds in the plants could be attributed to the shift from their natural habitat to cultivation conditions or other biological attributes. Other factors that could have contributed to the variations and differences in the compound content are the collection season, which spanned from April to October, the stage of the plants, altitude from where they have been collected and the extraction procedure. The study reveals that the plants growing in high altitude regions have been found to contain higher levels of phytochemical compounds compared to those growing at lower altitudes. This may be due to several factors like oxidative stress, cold temperatures, harsh weather etc.

In addition to this the several variants of plant extracts have shown to have antimicrobial activity. The antimicrobial activity was evaluated using disc diffusion method. The maximum antibacterial activity of ±29 mm and ±25 mm was found against *S. aureus* in acetone extract of Chopta and Acetone and methanolic extract of Tungnath accessions respectively. A clear zone of inhibition was also found against *Salmonella* of ±28 mm in Chloroform extract of Tungnath. The extracts did not show any activity against fungal species. The present study concluded that *Rheum* sp. extract have the great antimicrobial activity against *S. aureus*, *Bacillus.* Anthraquinone, a phenolic compound, is primarily responsible for the antimicrobial properties exhibited by the phytochemicals. These phytochemicals have the potential to be utilized in the management of various chronic diseases. Emodin, in particular, has been attributed to antibacterial activity, which is believed to occur through multiple mechanisms. These mechanisms include disruption of the bacterial cell membrane, inhibition of bacterial protein synthesis, and modulation of bacterial

biofilm formation. The antibacterial properties of emodin hold promise for the development of novel antibacterial agents for the treatment of bacterial infections. However, further research is required to evaluate its efficacy and safety in clinical applications.

Rheum, similar to numerous other medicinal plants, possesses primary metabolites such as carbohydrates and proteins, along with secondary metabolites including terpenoids, tannins, and saponins. These secondary metabolites are known to possess various medicinal properties such as anticancer, antifungal, and antiviral activities. Therefore, studying the phytochemical properties of *Rheum* can be beneficial for the pharmaceutical industry in developing new drugs to treat various ailments. The results obtained from this study can be valuable for the initial stages of drug development on a broader scale, potentially contributing to advancements in human health and well-being.

Based on the genetic data and phytochemical analysis obtained in this research, we propose diverse conservation approaches for efficiently managing this endangered species. To the best of our knowledge, this study represents the first report on genetic diversity and population genetic structure of *Rheum emodi* from Uttarakhand, utilizing RAPD and ISSR markers. Twelve RAPD primers amplified 142 fragments with an average of 11.8 fragments per primer and 65.64% polymorphic loci; additionally, 13 ISSR primers amplified 189 fragments in total, with an average of 14.5 fragments per primer and 62.84% polymorphism. Both RAPD and ISSR analyses revealed a low level of genetic diversity in the wild populations of *Rheum*. Furthermore, analysis of molecular variance (AMOVA) was employed to assess the variation within and between populations as well as between regions. The results indicated that the percentage of polymorphic bands (PPB) detected by ISSR and RAPD markers was almost the same. However, the study was unable to determine the superiority of markers and amplification reproducibility. Overall, this study provides valuable insights into the genetic diversity and population genetic structure of *Rheum emodi*, facilitating the development of conservation strategies for this endangered species.

If medicinal plants are systematically cultivated instead of being collected from the wild, many problems can be minimized. Growers can receive properly identified and certified planting materials, and cultivation can be planned to meet industry needs in the required quantities and at the required time, minimizing unintentional and deliberate adulteration. Wild plants are subject to soil, seasonal, and weather conditions, and may not be available year-round, making cultivation

more reliable. The present study aims to develop efficient procedures and facilities to obtain phytochemical and genetic data on large sample sizes for the authentication and detection of somaclonal variation. This work has not yet been done on this particular species in the northern region of India and will also contribute to conservation efforts.