Chapter 1

INTRODUCTION

Plant resources are the important gifts of nature to the mankind. Medicinal plants and their phytochemical are the basic raw materials for traditional and modern medicines and therapies. Plants are considered as the biosynthetic factories, producing a lot of secondary metabolites that confer them the medicinal properties. The secondary metabolites majorly comprise of alkaloids, flavonoids, tannins, phenolic compounds, saponnins etc. The healing characteristics of plants are linked to the existence of bioactive substances, whether occurring alone or in conjunction with other phytochemicals. These bioactive compounds are responsible for conferring the medicinal and pharmaceutical value to the plants.

The traditional medicines are used by around 60% of the world population not only for primary healthcare but been widely used in modern medicine system also. The plants are used as a source of medicines from prehistoric times. The Indian system of medicine encompasses various healthcare traditions that have originated from outside sources and assimilated into Indian culture. It comprises Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy.[1] India has the potential to become a significant hub for the pharmaceutical and phytochemical industries. Medicinal plants have been utilized for the treatment of ailments since the advent of modern scientific systems of medicine.

In modern times the use of the medicinal plants to cure illness has been increased due to the increased adverse drug reactions and cost of the modern medicines.[2] But due to the lack of clinical data and scientific studies that will provide better understanding of the well-being and effectiveness of the herbal drugs the modern system of medicines and Ayurveda drugs cannot be used side by side. However, various scientific theories regarding the effects and efficacy of medicinal plants is not well documented and we lack the knowledge regarding the genetic and physiological makeup of most of the herbal drugs. The need of the hour is the joint cooperation of Botanist, Biotechnologist, Pharmacologists and Phytochemist so that the various biosynthetic pathways leading to the formation of bioactive compounds or the secondary metabolites, and the active use of that active component that adds values to the plant can be known.

India is home to approximately 45,000 species of medicinal plants, with significant hotspots located in the Andaman and Nicobar Islands, Indian Himalayan Region, Western Ghats. These regions showcase a rich diversity of plant species that possess medicinal properties. With its immense biotic wealth, India is one of the biggest producer of medicinal herbs and has more than 7000 species reportedly used for food and medicinal purposes. Most of these plants are being over exploited in its natural habitat for the extraction of drugs. It will be wise to study about these species of local/indigenous medicinal/nutraceutical/commercially viable plants at their genetic and molecular levels along with the development of efficient management and conservation strategies to study genetic & phytochemical diversity. Altitudinal variation at genetic and phytochemical level can be exploited to select the different useful genotypes as cultivars to avoid variation in batch-to-batch extraction of drugs.[3] Globally, emphasis is laid on exploitation of natural resources and patenting of plants in developing as well as developed countries to generate databases on indigenous medicinal/nutraceutical and commercially valuable plants which can be used further for future reference.

Unplanned development and mismanagement of ecosystem led to over exploitation of medicinal and commercially important plants from scientifically non-managed natural resources. This not only resulted in shortage of various medicinal and commercially important herbs but also leads to the extinction of several species in their natural habitat. To come across the growing demand from industries for therapeutic plants, it becomes crucial to prioritize the conservation of these species. Attaining this goal can be accomplished through diverse approaches, including the farming of medicinal plants and the application of conservation methods (ex-situ and in-situ). These conservation efforts aim to ensure the ecological development of pharmaceutically important plant species and maintain their availability for future generations. Emphasis is also laid on cultivation of plants of wild form, instead of collection from wild would ensure their proper botanical identity, genetic and molecular improvement, quality of plant and continuity of supply. These medicinal and nutraceutically important plants in traditional and local healthcare practices provides valuable clues for new areas of research and biodiversity conservation strategies. The primary objective of this study was to delve into the genetic makeup and chemical composition of the plant in question. By doing so, the researchers aimed to provide valuable insights that would enhance our comprehension of the plant's medicinal attributes and potential uses within various commercial applications. An interpretation of genetic difference in condition, amount and pattern is of prime

importance in study of genetics. This fundamental knowledge is a basis of tests involving evolutionary hypothesis, but also of immense importance for phytochemical profiling, *in vitro* techniques for cultivation of effective germplasm upgradation and *ex situ* plant conservation & preservation strategies and authentication of the selected stands and clonal plant material. The importance and usage of high-altitude pharmaceutical plants in general/traditional healthcare system, provides clue to new frontier areas of research in plant biodiversity conservation is a well-established feature.[4]

In the priority area of natural resource conservation and preservation of plants in the wild and their systematic cultivation outside their natural habitat is gaining significant importance during the last two decades, because of the alarming rate of depletion of these plant resources inimical to the natural regeneration of species. Biodiversity conservation strategies and germ-plasm methodology are of immense importance in plant conservation and breeding programs.[5]

India, a country lying in a tropical zone is a cradle to many medicinal/nutraceutical and commercially viable plant species. Moreover, like other developing nations, bio-diversity of medicinal plants in India is in perilous state - due to interminable capitalization of these medicinal plants by pharma companies. The proposed problem involves studies on available material of various populations of *Rheum sp.* through molecular markers for the assessment of genetic variability and diversity with respect to the active components in its altitudinal region of distribution. The conventional approach to gathering this information may require a significant amount of time, potentially spanning several years, to yield comprehensive results. It is proposed to standardize and isolate genomic DNA from the individuals of different populations. Standardize PCR conditions for the multiplication of the specific segment/region of the genes that could generate the database of the species in regard to the molecular diversity that could be used for recommending the most diverse source for the plant breeding programs. Thus, there is an urgent need to counter and tackle the problems like volume of genetic difference available in natural population of *Rheum* species in their habitat[6] and distribution of this genetic and molecular variation in different topographical zones i.e. natural habitat of *Rheum sp*. in Uttarakhand Himalayas.

1.1 About the Plant *Rheum*

Rheum or rhubarb, is commonly grown for its edible stalks. The stalks are typically used in pies, jams, and other baked goods. The plant is a participant of the Polygonaceae family and naturally belong to Asia. It is a perennial plant that can grow up to 5 feet tall. The leaves of the plant are toxic and should not be consumed as they contain oxalic acid which can be harmful in large quantities. Nevertheless, the stalks are safe for consumption and serve as a rich source of vitamin K, vitamin C, and dietary fiber.[7]

The term "Rhubarb" has its origins in the *Latin* words "rha" (river) and "barb" (barbarian land). During ancient times, the Romans imported the roots of rhubarb from lands beyond the Volga or Rha River, which were considered as barbarian lands. Additionally, the name is linked to the Greek word "rheo," which means "to flow," referring to the root's purgative properties, as stated in Lindley's Treasury of Botany. In India, the International Union for Conservation of Nature (IUCN) has listed a total of 560 plant species, including *Rheum* species, in the Red List of Threatened Species. *Rheum emodi*, in particular, is classified as critically endangered, making it a high-priority species for conservation and cultivation efforts.[8] The export of *R. emodi* is either prohibited or requires a special export permit due to its endangered status.[9]

Rhubarb is frequently used medicinal plant usually called as the “Wondrous drug” as it possesses a lot of therapeutic actions. Genus *Rheum* consist of a total of 60 species found around the world out of which only 10 species are found in the Indian Himalayan Region. Some species are *R. emodi, R. webbianum, R. austral*, etc. The plants belonging to family Polygonaceae possess a chromosome no. x = 11 and can be diploid, tetra-ploid and hexa-ploid. The rhizome of plant is well known for its therapeutic actions and is also grown as ornamental shrub. *Rheum emodi* also known as the “Himalayan Rhubarb” is the main *Rheum* species found in India and is also officially labelled in the Indian Journal of Pharmacopeia. *Rheum* species is an herbaceous plant growing with fleshy roots. The plant is characterized by an upright growing stem and basal leaves. These leaves are deciduous and extend from short, broad rhizomes that have a deciduous core. The arrangement of flowers on a stalk are terminal and loose branching clusters-like pedicels. The flowers are hermaphrodite consisting whitish to pinkish green, campanulas and hairless sepals and petals (six tepals).[10] Many *Rheum* species have been utilized for nutraceutical purposes since their origins in Asia nearly 2,000 years ago. *Rheum* plant has high concentrations of oxalic acid, a toxic

compound, but the concentration of this compound in the parts used for food preparation, such as the leaf stems and petioles, is very low. The non-toxic malic acid imparts the tart flavour to the plant which gives it a unique taste. Other than oxalic acid, some poisonous compounds like citric acid and anthraquinone-based glycosides are also produced by the plant. If consumed in large amounts, both raw and cooked leaf blades can be toxic to animals and humans. It is important to exercise caution and ensure proper cooking or processing methods are employed to minimize the toxicity of the plant. *Rheum* species can also be propagated by seeds and cutting up the crowns of large plants.[11]

1.2 Origin and Distribution of species

From ancient times, *Rheum* plant has been used to cure various health related ailments documenting its use in Chinese medicine in 2700 BC. During the first century it was imported for medicinal purpose in Greece and Rome. *Rheum*, is believed to have originated in China and parts of Siberia. The plant has been cultivated in Asia, spanning thousands of years. It has been utilized for both culinary and medicinal purposes during this time. Rhubarb was brought to Europe in the 18th century and quickly became popular as a food ingredient. The cultivation of the *Rheum* plant has expanded beyond its Asian origins and is now widely practiced in various parts of the world. It is cultivated in regions across Europe, North America, and parts of South America where the cool and temperate climate conditions are suitable for its growth. Nowadays rhubarb is cultivated in other parts of the world as well, including Australia and New Zealand. This global cultivation highlights the adaptability of the plant to different climates and its popularity among farmers and gardeners worldwide. It is profoundly grown in many other countries like Russia, Canada, Poland, and Germany. The plant is also found in the wild in some regions, such as in parts of Asia and Europe.[12]

The medicinal purpose of *Rheum* was brought up in India around 10th Century along with its spread to Russia, North America and Europe.[13, 14] *Rheum* is a short herbaceous plant, which is endemic to Himalayan region distributed from Kashmir to Sikkim. The plant is not native to India, and its cultivation is not widespread in the country. While India has a rich diversity of medicinal plants, the cultivation of *Rheum* is relatively limited in comparison to other regions. However, it is grown in some regions, particularly in the cooler, higher altitude areas of the Himalayas, such

as in Himachal Pradesh, Uttarakhand and parts of Kashmir.[15] The plant has the ability to thrive in different soil compositions and necessitates a climate that is both cool and humid.

The plant species is found in the wild in few parts of Uttarakhand state, India as the state is situated in the Western Himalayas and has a cool and temperate climate, which is suitable for growing rhubarb. The plant is commonly found in the higher altitude regions of the state, such as in the mountainous districts of Pithoragarh, Chamoli, and Uttarkashi and in some areas of the Kumaon area of Uttarakhand, which is known for its rich biodiversity. According to Badoni *et al.*, (2009) the local communities utilize the plant for its medicinal and culinary benefits.[16] Rhubarb is traditionally used in the state for medicinal purposes and is considered a valuable plant by local communities. The plant is used to make various Ayurvedic and traditional medicine preparations.

1.3 Potential Benefits

Rheum, also known as rhubarb, has a number of potential benefits. [17] Some of these include:

- Digestive health: Rhubarb is a natural laxative and can be used to relieve constipation. It is also believed to help stimulate the production of bile, which can help improve digestion.
- Anti-inflammatory: Rhubarb exhibits anti-inflammatory properties and shows potential for treating conditions like osteoarthritis and rheumatoid arthritis.
- Cancer prevention: According to some studies compounds found in rhubarb may have anti-cancer properties and is useful in the inhibition of certain types of cancer.
- Antioxidant: Rhubarb is abundant in antioxidants, which play a starring role in safeguarding the body contrary to harm caused by free radicals. Furthermore, these antioxidants may potentially lower the likelihood of developing specific chronic ailments.
- Blood sugar control: Rhubarb may be helpful in the management of diabetes. It is low in sugar, and some studies have suggested that it may help to lower blood sugar levels. [18]
- Heart health: Rhubarb is characterized by its significant potassium content, which contributes to the potential of lowering blood pressure and diminishing the threat of heart disease.

Further research is required to fully understand the potential benefits of *Rheum* and to confirm its effectiveness and safety. Due to the numerous medicinal properties associated with *Rheum* species, there is a significant demand for these medicinal plants, leading to unregulated collection by

pharmaceutical companies seeking their active components. Additionally, local communities heavily rely on these herbs for domestic use and traditional herbal mixtures, exerting significant pressure on the availability of these plants.

1.4 Ethnobotanical Information

Ethnobotanical information can be used to understand the ecological relationships between humans and plants, and to develop sustainable land-use practices. By studying the traditional uses of plants, scientists can learn about the ways that different cultures have managed their environment and how they have used plants in a sustainable way. *Rheum* is used in traditional medicine systems from a long time. [19] The Insights into the interactions between plants and humans can be utilized to formulate conservation strategies and promote the sustainable utilization of plant resources.

In traditional Chinese medicine, rhubarb root is used in treatment of a variety of digestive problems, like constipation and diarrhoea. Furthermore, it is employed in the cure of numerous skin conditions, including eczema, and is known to aid in reducing fever.

In Ayurveda, rhubarb root is used to treat digestive problems, such as constipation and diarrhoea. It is also used to help relieve pain and inflammation in conditions such as osteoarthritis and rheumatoid arthritis.

In Europe, the root of rhubarb is traditionally being used as a laxative. It is also believed to hold potential in treating conditions like gout and rheumatism.

In the Himalayan region, rhubarb is used as a traditional medicine, particularly in the treatment of digestive disorders. In addition to its applications in treating fever, the plant is also utilized for addressing ailments such as intestinal worms and various other health conditions.

In the Kumaon region of Uttarakhand, India, rhubarb is traditionally used for medicinal and culinary purposes.

1.5 *Rheum*: Nutraceutical Uses

According to Nazir *et al.,* (2013), rhubarb exhibits potential nutraceutical uses,[20] including:

- Abundance of dietary fiber: The stalks of rhubarb are an excellent source of dietary fiber, promoting healthy digestion and regular bowel movements.
- Significant vitamin K content: They serve as an excellent source of vitamin K, a crucial nutrient essential for blood clotting and maintaining bone health.
- Vitamin C provision: The stalks of rhubarb also provide a notable amount of vitamin C, an antioxidant that boosts the immune system.
- Rich in antioxidants: Rhubarb contains a variety of antioxidants such as polyphenols, flavonoids, and anthocyanins, which help protect the body against free radical destruction and potentially decrease the risk of certain chronic diseases.
- Low sugar content: *Rheum* have a very low sugar content, making it a suitable food choice for individuals with diabetes or those seeking to manage their blood sugar levels.
- Laxative properties: Traditionally, rhubarb root aid in constipation and digestive disorders and been used as a laxative.

1.6 Phytochemistry

Phytochemicals are chemical compounds that are naturally found in plants. They are not essential for human survival, but they have been shown to have various health benefits when consumed as part of a balanced diet. several studies suggest that phytochemicals, which are natural compounds found in plants, help in reducing the risk of chronic diseases like cancer and coronary disease. Secondary metabolites, are released by the plants as a result of their metabolic processes. Phytochemicals also serve a vital role in protecting plants from herbivores, pathogens, and various environmental stresses. These chemical compounds act as defence mechanisms, deterring potential threats and ensuring the plant's survival. Additionally, phytochemicals participate in plant-to-plant communication, allowing them to interact and respond to their surroundings, including neighbouring plants. Examples of secondary metabolites include alkaloids, terpenes, and flavonoids. Some compounds can be both secondary metabolite and phytochemical. For example,

flavonoids can be found in both categories, as they can be secondary metabolite but also have a protective role for the plant and potential health benefits for humans.

Rheum, contains a variety of phytochemical compounds.[21] Some of the most notable phytochemicals found in rhubarb include:

- Anthraquinones: Rhubarb root contains a group of complexes called anthraquinones, responsible for its laxative effect. These compounds stimulate the muscles in the intestines, helping to promote bowel movements.
- Tannins: Rhubarb contains tannins, which are composites that have astringent properties and can aid to decrease inflammation.
- Polyphenols: Rhubarb contains various polyphenols such as flavonoids, anthocyanins, and catechins which are known for their antioxidant properties.
- Oxalic acid: Rhubarb leaves contains high levels of oxalic acid which can be harmful if consumed in large amounts, so it is important to avoid eating them.
- Saponins: Rhubarb root contains saponins, which are mixtures that have anti-inflammatory and anti-cancer properties.
- Vitamin K and Vitamin C: Rhubarb stalks are rich in Vitamin K and Vitamin C.
- Flavonoids: Rhubarb contains flavonoids, which are a class of antioxidants that have shown to have Anti-inflammatory and Anti-cancer properties.
- Anthocyanins: Rhubarb also contains anthocyanins, which are pigments that give the plant its red color and also have antioxidant properties.
- Catechins: Rhubarb also contains catechins, which are a type of flavonoid that possess Antioxidant and Anti-inflammatory properties.

Emodin, aloe-emodin and rhein are the phytochemically important compounds that are found in the roots of rhubarb, also known as *Rheum.* These compounds are together classified as anthraquinones.[22]

- Emodin: Emodin is an anthraquinone that is believed to stimulate the muscles in the intestines, helping to promote bowel movements. It also has potential anti-cancer, anti-inflammatory, antioxidant, anti-viral, and anti-bacterial properties.

- Aloe-emodin: Aloe-emodin is structurally similar to emodin and it is also found in the roots of rhubarb. It has been found to have similar properties as emodin and it has been investigated for its potential anti-inflammatory, antioxidant and anti-cancer properties.
- Rhein: Rhein is an anthraquinone glycoside found in rhubarb, it is a natural laxative and has been traditionally used to treat constipation and diarrhea. It also has anti-inflammatory and antioxidant properties and has been investigated for its potential use in the cure of osteoarthritis, cancer and other diseases.

1.7 Methods for Assessment of Genetic Diversity

Genetic diversity studies aim to understand the genetic variation within a population of organisms including plants, animals, and microorganisms. The goal of these studies is to identify genetic variation within a population, as well as to understand how that variation is distributed and how it is related to other factors such as environment, adaptation, and evolution.[23]

In plants, genetic diversity studies can help to identify genetic variation within a species or population of plants, which can be useful for breeding programs, conservation efforts, and understanding the evolution of the species. We can carry out these studies using various molecular markers, including but not limited to Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR), and Single Nucleotide Polymorphism (SNP) markers. [24, 25, 26]

1.7.1 Biochemical Markers

In genetic diversity analysis, there are multiple types of biochemical markers that can be employed, such as:

- Enzymes: Enzymes are proteins that catalyze chemical reactions. Enzymes are commonly used as biochemical markers in genetic diversity studies because they are easy to extract and analyze. Enzyme-based markers are also known as allozymes.[27, 28]
- RAPD: Random Amplified Polymorphic DNA (RAPD) markers are based on the “Polymerase Chain Reaction” (PCR) which amplifies random fragments of DNA. These markers are simple, rapid and economical and can be utilized to study GD in a wide range of organisms.

- ISSR: Inter Simple Sequence Repeat (ISSR) markers are based on PCR amplification of regions between simple sequence repeat (SSR) sequences. These markers are useful in detecting variations in the number of SSRs between different individuals or populations.[29]
- AFLP: Amplified Fragment Length Polymorphism (AFLP) markers rely on selective PCR amplification of restriction fragments. AFLP markers are sensitive and have a high level of resolution, making them useful in detecting genetic variation in populations.[24]
- SSR: Simple Sequence Repeats (SSRs) markers, or microsatellites, are short repeat sequences of DNA. SSRs are highly variable and used to detect "genetic variation within and between populations".
- SNP: Single Nucleotide Polymorphism (SNP) markers work by the detection of single base differences in the DNA sequences. These markers are highly specific and are useful in identifying genetic variation and population structure.[30]

The different markers have different characteristics and a combination of markers can be used to increase the resolution and accuracy of the analysis.

1.8 Research Hypothesis

Molecular studies are necessary for planning and designing conservation and management strategies as well as in selection of diverse germplasm lines for cultivation and further improvement of the species in future. The current study aims to develop efficient routine procedures and facility for obtaining genetic data on large number of samples in order to specify and detect the variation among the distinct geographical sources through molecular markers, which will be used for the authentication of the plant material. The study focusses to look in depth the genetic and molecular difference of the medicinal plant material that are used in state of Uttarakhand by local population for the various ailments.

Rheum species are of immense medicinal importance, because of unsustainable harvesting, habitat loss, deforestation, grazing pressure and other biotic pressures, the wild populations of *Rheum* have become very rare and sparse. The plants growing in wild conditions are dependent on soil, seasons and weather conditions, hence they may not be available throughout the year. Thus, there is a need to address the issues like level of genetic variation present in natural populations of *Rheum* species and how this genetic variation is distributed in different geographical regions i.e.

population structure of *Rheum* species in Garhwal Himalaya. The plants can also be made available by mass in vitro clonal multiplication of the plant species.

Thus, in the proposed study the available material of various populations of *Rheum sp.* will be evaluated through molecular markers for the assessment of genetic variability and diversity with respect to the active components in its altitudinal zone of distribution. The isolation protocol for genomic DNA from the individuals of different populations will be standardized and PCR conditions for the multiplication of the specific segment/region of the genes that could generate the database of the species in regard to the molecular diversity will also be focussed upon. The generated data could be used for recommending the most diverse source for the plant breeding programs. No study on the genetic diversity and population structure at the molecular level is reported from Garhwal Himalayas till date.

Keeping into consideration the above research hypothesis and research gap, the present study is focussed on following objectives.

The objectives are:

- To analyze the effect of microbial/chemical elicitors on the structure and yield of the active constituent.
- Standardization of DNA isolation, purification, optimization of PCR conditions for the specific gene regions with the objective of screening various populations of *Rheum sp.* for polymorphism and genetic analysis.
- To document and analyze patterns of genetic variability and diversity within and among populations of *Rheum sp.* with the help of molecular genetic markers (DNA).