Chapter 2

REVIEW OF LITERATURE

Medicinal plants have been used for thousands of years to treat a wide variety of ailments and conditions. They have played a critical role in the development of modern medicine and are an important source of natural remedies for people worldwide. Medicinal plants are an important component of global biodiversity and help maintain ecosystem health. Many present day medicines are derived from natural compounds found in medicinal plants, making them an important source of new drugs and treatments. They can be grown sustainably, providing a renewable source of natural remedies and offer a range of benefits making them an important area of research and development from ages.

Uttarakhand is a state in northern India known for its rich biodiversity, including many species of medicinal plants.[31, 32] Some of the pharmaceutically important plants found in Uttarakhand are Himalayan Yew (*Taxus wallichiana*), Kutki *(Picrorhiza kurroa*), Rhubarb *(Rheum emodi*), Kalmegh (*Andrographis paniculata*), Amla (*Emblica officinalis*), Bael (*Aegle marmelos*), Ashwagandha (*Withania somnifera*), Fagopyrum, Jatamansi, etc naming a few. The present review includes different aspects of *Rheum* plant like its origin, ethnobotanical data, pharmacological importance, genetic diversity analysis studies etc.

2.1 Experimental Plant

Rheum species belongs to the family Polygonaceae, basically is a robust perennial herb. There are approx. 60 species of the *Rheum* genus found around the world, while only 10 species found in the Indian Himalayan region.[33] These *Rheum* species have a consistent chromosome number of 2n = 22, with a fundamental set of 11 chromosomes, and the karyotype formula is 2n = 22 (20 metacentric + 2 submetacentric).[34]

Rheum is a genus of perennial plants commonly known as rhubarb. Within this genus, several species are cultivated for their edible stems, which are thick and fleshy, often used as a vegetable.

The stem is usually red, green, or pink in colour, depending on the species. The roots are important pharmaceutically and is often used for medicinal purposes. It is typically yellow or brown in colour and has a bitter taste. *Rheum* plants are known for their large, fleshy stems and triangular leaves.[35]

Rhubarb, also known as Dolu, is recognized by different names in different geographical regions across the world and in different languages. In common English language *Rheum emodi* is called as Indian Rhubarb or Himalayan Rhubarb. In Sanskrit as *Revatchini*, in Hindi as Dolu, Arabic as Rewand, in Persian as Rewandchini. In ayurvedic system the plant is called as Amlaparni, Gandhini Revatikka and in Unani System of Medicine as Revandchini. The parts of plants of *Rheum* species used for medicinal purpose are roots and rhizomes are very expensive as the plant is very rare and is under endangered category.[10] The classification of *Rheum* taxonomically is given in Figure 2.1.

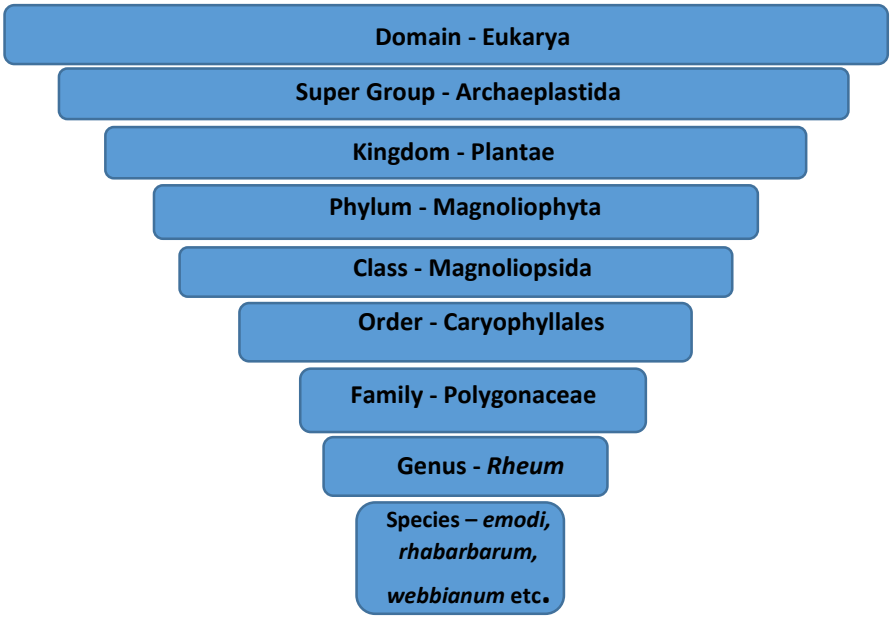


Fig 2.1: Taxonomic Classification of *Rheum*

2.2 Origin and Distribution of Species

The commercially known types of Rhubarb, referred to as Chinese, Russian, Turkish, and East Indian, are said to be derived from species *R. officinale and R. palmatum. R. officinale* is found extensively in central and western China at an altitude of 3,000 to 4,000 meters[36], while *R. palmatum Linn*. occurs in the thrives province of Kansu sub-alpine and alpine Himalayas, at 11,000-12,000 ft.[37,38] *Rheum* is dispersed in the regions of "Himalaya, Kashmir, Sikkim, Uttarakhand and Himachal Pradesh"[39] grown in an elevation of 2800- 4000 meter. *R. emodi* grows in the wild in the mountains of the "Western and North-western provinces of China, as well as in the Tibetan territory". It is also found growing wildly in various parts of Nepal and Sikkim to Kashmir, at altitudes ranging from 4,000 to 12,000 ft[2]. Additionally, *R. emodi* is cultivated in Assam. On the other hand, *R. palmatum* is grown in a long chain of mountains, partly with naked forests, extending from the western part of Chinese Tartary to the north near the town of Selia and as far south as Lake Kokanore, near Tibet. Moreover, it is grown in the regions of Europe and the United States.

Rhubarb grows easily in cool, temperate climates on high altitudes in Asia from Tibet to Southeast China. The drug is obtained from both wild and cultivated Rhubarb, and it has been successfully grown in certain parts of Assam.[40] In Uttarakhand Himalaya ranges it is found in the altitude ranging from 3300-4000m. *Rheum* is available in various regions, including Pothivasa at an altitude of 2,000 meters, Malari at 3,400 meters, Mana at 3,450 meters, and Tungnath at 3,600 meters in district Chamoli, Uttarakhand, India.[41] There are approximately 10 species of Rhubarb found in the temperate and alpine regions of the Himalayas. However, only two of these species, *Rheum emodi Wall* and *Rheum moorcroftianum Royle,* are cultivated in the Garhwal region of the Himalayas.[42]

2.3 Morphological Characteristics

Rheum is a perennial herb that has thick, sturdy roots and a hollow stem. The leaves are large, reaching up to 40 cm in length and having a broad ovate shape.[21,43] The flowers are dark, reddish-purple or pale red, bloom in June and July in axillary panicles that appear in large clusters. The roots and rhizomes which have most of the medicinal usage are harvested in autumn, usually in

late September when they are around 8-10 years old. The rhizome is the most important part of the plant used for drug preparation. After removing the bark, the stems are either cut into tiny pieces or segments and dried in the sun/kiln. The solid, compact, and heavy rhizomes of *R. emodi wall* are cylindrical or barrel-shaped.[37] The rhizomes have brownish or yellowish-brown covering whereas the inner part is yellow in colour. They are aromatic and slightly pungent with a bitter and astringent taste and have a strong, peculiar, and faintly aromatic fragrance. When chewed, they feel gritty under the teeth. Seeds are usually oval in shape and collected in late August–September, when they turn dark brown. Seeds show poor germination rate. The plant has juvenile phase of three to five years after that reproductive phase occurs. Various morphological characteristics of *Rheum* plant are illustrated in Figure 2.2.

2.4 Major *Rheum* Species

Genus *Rheum* consist of a total of 60 species found around the world out of which only 10 species occur in the Indian Himalayan region. Some species are *R. emodi, R. webbianum, R. austral,* etc.

Rheum emodi is a recurring plant that propagates automatically from sturdy, small rhizomes that stand upright when season comes. Its sizable leaf blades are somewhat triangular in shape and are supported by lengthy petioles. The plant produces slight greenish-white coloured flowers in large inflorescences made up of compound leaves. This herbaceous perennial plant has green streaks and its leaves are orbicular or broadly ovate. The petioles are long and robust, while the inflorescence forms panicles and the flowers are dark purple. Its sepals number 5, while its stamens range from 6-9 and the fruit is oblong and purple, measuring long. This plant belongs to a family that comprises over 800 species and 40 genera, all of which have jointed stems. From ancient times *Rheum emodi,* (Himalayan Rhubarb) is used as a medicinal herb in Ayurvedic system of medicine to cure a lot of diseases. Rhubarbs are large plants with solid or hollow flower stalks that reach a height of 1-2 meter. The leaves are radical that form a rosette which are very large with long, succulent petioles. The plant is cultivated using seeds, seedlings, or pieces of rhizome.[44]

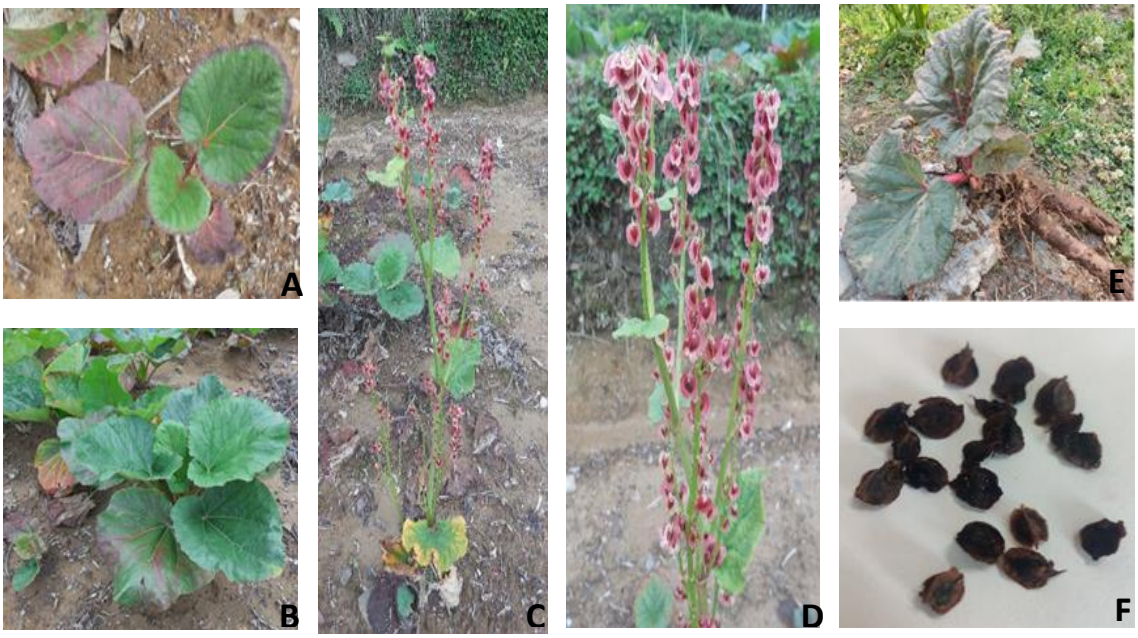


Fig 2.2: Morphological Characteristics of *Rheum* at different stages

A = Juvenile young plantlet, B = Young plant, C = Matured plant, D = Flowers, E = Rhizome, F = Seeds

Rheum webbianum Royle is a significant medicinal plant native to Tropical regions, spanning from China to India, Nepal, and Pakistan. In India, it is commonly found in Himachal Pradesh, Jammu and Kashmir, and Uttar Pradesh. The leaves are generally large, ovate, measuring around 30-60 cm in length, and are pubescent on the abaxial side and papilliferous or muricate on the adaxial side. The species grows up to 1.5-2 meters tall, occasionally up to 2.5 meters, and has a stout, straight root that measures around 30 cm (length) and 10 cm (diameter). The root or rhizome is dark brown from outside and yellow or red-yellow coloured from inside. The flowers are greenish-white to rose-red in colours and are grouped in large leafy inflorescences.[45]

Rheum spiciforme is a strong plant that has thick petioles on its basal leaves that are purplish red in color. The leaves are broadly elliptic and leathery, and have papilliferous surfaces and glabrous abaxial surface. The leaf blade is purple-red on the abaxial surface and dark green or yellow-green on the adaxial surface. All leaves are radical, and have a very hard petiole that is usually glabrous. The plant's inflorescence is pedunculate, glabrous, and measures around 10-30 cm long in a spike-

like raceme. The flowers are small, measuring 2-3 mm across, and have a pedicel that is 2-7 mm long.[46]

Rheum moorcroftianum is differentiated from other *Rheum* species on the absence of stem. Basal leaves are present at the base which provide them their unique identity. Inflorescence is generally branched panicle, anthers are pinkish-red and seeds are red in colour which when matured turn brow and often 3-winged.[41]

R. australe is a robust, tall perennial herb with a leafy appearance. The stem can be either glabrous or pubescent and has a green and brown streaked colour with shades of purple and red. The rhizomes of the plant are untrimmed, coarse, and have yellowish brown surface. The leaves can grow up to 60 cm in diameter and are highly wrinkled with a rough surface, thick, dull green, and orbicular or broadly ovate in shape. The flowers are dark red-purple and densely branched.[19] Propagation is possible through seeds or rootstocks. When sown immediately after harvesting the mature sees show high germination rate, otherwise the shelf life of seeds is very low. [47]

2.5 Pharmacological Actions and Potential Benefits

The medicinal benefits of the plant are well-known and many species of *Rheum* have nutraceutical value. The *Rheum* species has its origins in Asia dating back almost 2,000 years. The tart flavour associated with this species is due to malic acid in the plant parts. The *Rheum* plant also produces other poisonous compounds such as anthraquinone-based glycosides and citric acid. When consumed in large quantities the leaf blades are poisonous to animals and humans due to the presence of oxalic acid. *Rheum emodi* acts as diuretic, laxative, stomachic, and astringent tonic. Root possess anti-inflammatory, anti-dysentery activity and used as expectorant, appetizer and can be used as a preservative against inflammatory diseases.[48]

Rhubarb is used in various traditional systems of medicines (UNANI, Ayurveda, Chinese) from ancient times. The different part of plants is used in the treatment of diarrhoea and digestion related issues in children. Root is widely used in home remedies to treat stomach problems, cuts, wounds, muscular swelling, tonsils, mumps etc.[49] Root powder is used to cure mouth ulcers and for teeth cleaning. When mixed with honey the root powder is often used to treat cough. Stems, leaf and flowers are used as vegetables, the leaves especially in Uttarakhand region are used for making sauces because of their tarter taste. The leaves and stems are used for making pickles, jams and

pie all over the world. Besides all the medicinal properties, the stems are used in coloration in textile and wooden industries.[50] Apart from all the pharmaceutical uses, a yellow dye obtained from the rhizomes of *R. australe* is used in cosmetics and for coloration of hair, textiles and wooden materials.[8]

R. rhaponticum is utilized in the past to treat various stomach related health issues, like gastrointestinal pain, gastritis, liver and spleen disorders, pericardium-related heartache, and respiratory system dysfunctions. It was also used for reproductive system disorders like uterine and breast pains. A vinegar macerate was used to treat skin conditions like itching and scratches, while a water macerate was suggested for ulcers and to purify the blood. In addition, it was believed to have the ability to stop bleeding, alleviate fever, and cure injuries, trauma from falls, and vein disorders, according to certain fragments of historical sources.[51]

2.6 Phytochemistry

Rheum species contains various phytochemicals such as stilbenes, anthraquinones, ethers and esters, anthrones, phenols, carbohydrates, oxalic acid, oxanthrone, flavonoids and lignans. Among these, anthraquinone and stilbene are the most common constituents found in the plant. The anthraquinones include emodin, aloe-emodin, chrysophanol, physcion, chrysophanein, and emodin glycoside, while the stilbene group consists of resveratrol, picetannol, and their glycosides.[52] Oxanthrone derivatives like oxanthrone ether, oxanthrone esters, and revandchinone-3 are also present.

Currently, a lot of phytochemically active components are identified and characterized from various species of *Rheum*. Rhubarb is a significant herb in traditional Chinese medicine, and its constituents have been categorized into five major groups: Anthraquinones and anthrones, stilbenes, flavonoids, essential oils and sterols.

2.6.1 Anthraquinones and Anthrones

Anthraquinones contain a 9,10-anthracenedione skeleton (Figure 2.3). The diverse biological activities of *rheum* species including anticancer, antimicrobial, and anti-inflammatory properties are due to the presence of Anthraquinones. They are also commonly used in the production of dyes, as they exhibit strong and vibrant coloration. In the context of traditional medicine,

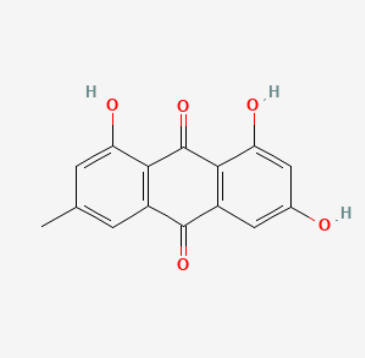
anthraquinones are often used as laxatives due to their ability to stimulate bowel movements. Examples of free anthraquinones are rhein, chrysophanol, emodin, and aloe-emodin, which are commonly found in most of the rhubarb species.[53] Apart from Polygonaceae family, anthraquinones and anthrones are also present in the members of family like Rubiaceae, Liliaceae, Fabaceae, Bigoniaceae and Verbenaceae.

- **Emodin**: Emodin is a natural organic compound that belongs to the anthraquinone family. According to researchers emodin is found to cause programmed cell death in human breast cancer cells by lowering the activity of Bcl 2 protein and accumulating the activity of caspase-3, PARP, p53, and Bax proteins.[54] It also blocks the growth and division of MKH45 cells in the human gastric cancer cell line by causing them to pause or stop at either the G2/M stage of the cell cycle.[55]

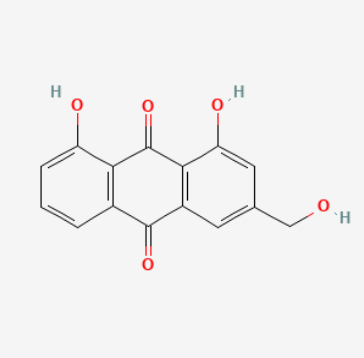
- **Aloe-emodin**: Research indicates that aloe-emodin can hinder the expansion of lung squamous cell carcinoma cells in the CH27 cell line. This is achieved by enhancing the presence of Bcl-2 family proteins and initiating the activation of caspase 3, caspase 8, and caspase 9.[56] Likewise, it impedes the growth of gastric cancer cell lines like MKH45 by causing a pause in the cell cycle during the G_o/G_1 phase.[55]

Aloe emodin and emodin are anthraquinones with empirical formula $C_{15}H_{10}O_5$ and molecular weight 270.24 g/mol. Melting point of aloe emodin found out to be 224°C and that of emodin was 257°.[57]

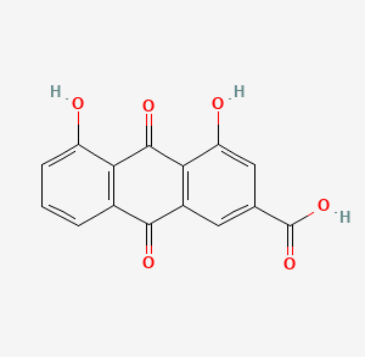
- **Rhein**: It triggers apoptosis in COLO 32DM colon cancer cells as well as in tongue cancer cells, nasopharyngeal carcinoma cells, and promyelocytic leukemia cells.[58,59]
- **Chrysophanol**: It has role in non-programmed cell death in a human liver cancer cell line through necrosis. It triggers mitochondrial dysfunction, assembly of Reactive Oxygen Species (ROS), DNA damage, promotion of LDH activity, and depletion of ATP, resulting in necrosis of the cells.[60]
- **Physcion**: It triggers programmed cell death in HeLa cells through the activation of various proteins including p21, p53, Bax, Bcl2, caspase-9 and caspase-3. This mechanism has been utilized as an anticancer drug against cervical cancer in humans.[61]



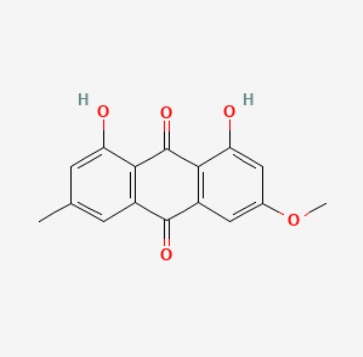
Emodin



Aloe- emodin



Rhein



Physcion

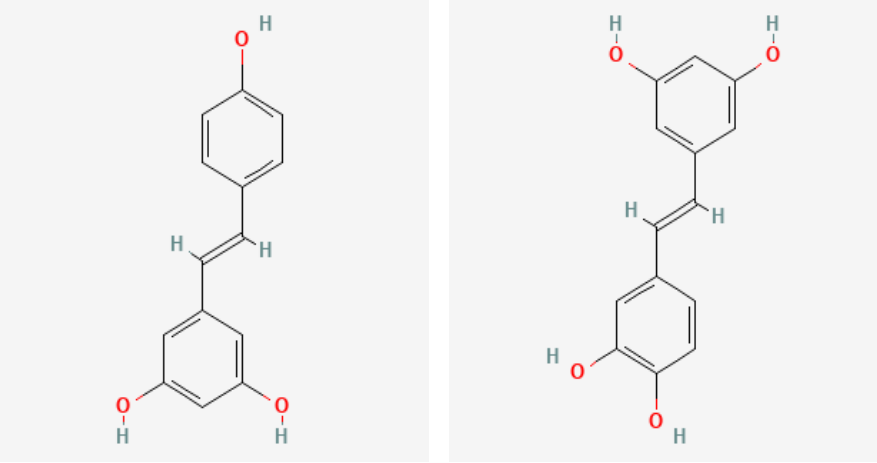
Fig 2.3: Chemical structure of Anthraquinones present in *Rheum* species

Structure source: PubChem

2.6.2 Stilbenes

Stilbenes are polyphenolic compounds that are found in various plants consisting of two aromatic rings connected by an ethylene bridge (Figure 2.4), and some examples of stilbenes include resveratrol and piceatannol. They have anti-oxidant properties and widely studied for potential health benefits, including anti-inflammatory, anti-cancer, and cardioprotective effects. These compounds possess various biological activities such as inhibition of nitric oxide production, anti-bacterial and anti-fungal effects, antioxidant and anti-inflammatory properties, as well as anti-cancer and anti-malarial activities.

- **Resveratrol:** The presence of resveratrol in rheum leads to various beneficial properties such as anti-cancer, antioxidant, anti-atherogenic, antimicrobial, anti-inflammatory, and estrogenic effects. Additionally, it is used for treating conditions related to cardiovascular and neurodegenerative diseases.[62]
- **Piceatannol:** Inhibiting adipogenesis, lowering lipid accumulation, lowering blood sugar, and attenuating oxidative stress and inflammation are only a few of their effects on metabolic disorders.[63]
- **Rhaponticin:** It is responsible for Anti-oxidant, anti-bacterial, antipyretic, anti-inflammatory, anticancer activities.[64]
- **Rhapontigenin:** They act as Anti-allergic, anti-oxidant, anti-inflammatory, anti-cancer and anti-coagulant.[65]



Resveratrol **Piceatannol**

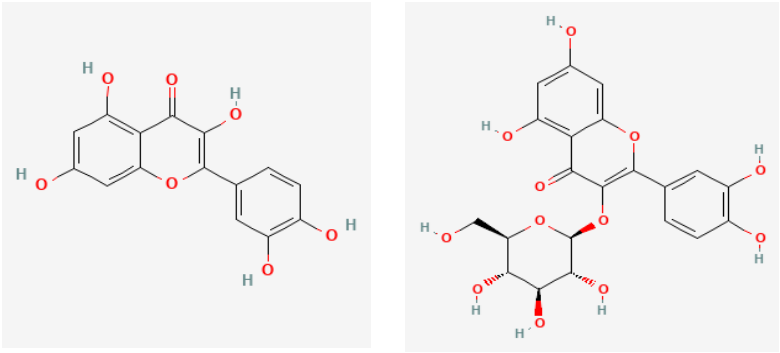
Fig 2.4: Chemical structure of Stilbenes present in *Rheum* species

Structure source: PubChem

2.6.3 Flavonoids

Flavonoids are characterized by their chemical structure, which includes a flavone nucleus with 2 benzene rings connected by a 3-C bridge (Figure 2.5). Flavonoids, which are abundant in various species of rhubarb, are compounds that possess multiple phenolic hydroxyl groups attached to ring structures, making them potent antioxidants.

- **Quercetin**: Acts as an antioxidant by reducing oxidative stress in the body's tissues. It also has a beneficial effect on the cardiovascular system.[64]
- **Isoquercetin**: Exhibits antineoplastic activity, anti-oxidant
- **Rutin**: The Antioxidant, antimicrobial, antifungal, anti-allergic agent, neuroprotective, vasoprotective and cardioprotective activities in plants is due to the presence of rutin.[66]



Quercetin **Isoquercetin**

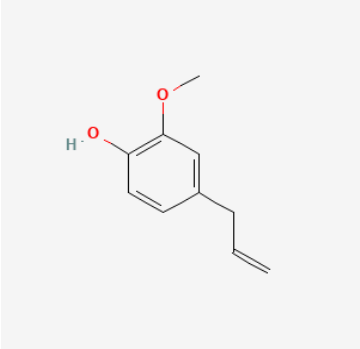
Fig 2.5: Chemical structure of Flavonoids present in *Rheum* species

Structure source: PubChem

2.6.4 Essential Oils

Essential oils are hydrophobic liquids that contain volatile chemical compounds derived from plants (Figure 2.6).

- **Eugenol:** They have Antiseptic, anti-inflammatory, anti-oxidant, anti-oxidant, antifungal, neuroprotective activities.
- **Paeonol**: Anticancer, anti-allergic, anti-inflammatory and aids in protection of cardiovascular system.
- **Methyl stearate**: It has a role as a metabolite. They act as antifoaming agent and fermentation nutrient.
- **Methyl eugenol**: Anti-anaphylactic, anti-allergic and anti-inflammatory effects.[67]



Eugenol

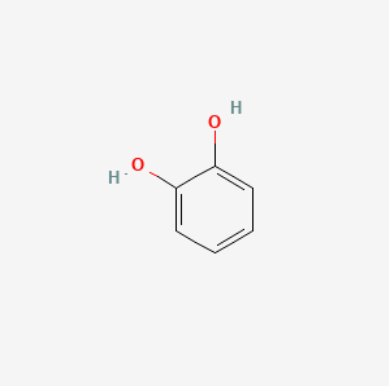
Fig 2.6: Chemical structure of Essential oils present in *Rheum* species

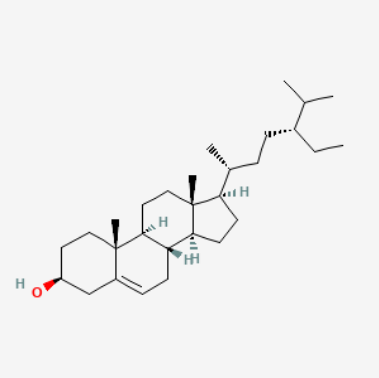
Structure source: PubChem

2.6.5 Sterols and Polyphenols

Sterols are a type of organic molecule that contains a specific arrangement of carbon atoms, including (-OH) group (hydroxyl group) attached to one of the carbon atoms (Figure 2.7). Sterols are present in foods derived from plants and have been connected with advantages for health, such as the reduction of cholesterol levels. Polyphenols form a broad and varied category of naturally existing substances discovered in numerous plant-based foods like fruits, vegetables, tea, and cocoa. Their reputation lies in their anti-oxidative characteristics, and they have been associated with various health advantages, encompassing the mitigation of risks tied to persistent illnesses like heart disease and cancer.

- **β- Sitosterol:** Anti-inflammatory, induce apoptosis, chemoprotective, hypocholesterolemic activity, immunomodulatory activities.[68]
- **Daucosterol:** Involved in development, apoptosis, proliferation, carcinogenesis and tumor growth.[69]
- **Epicatecholgallate:** Powerful antioxidant, preventing oxidative damage in healthy cells, and act as antitumor agent.[70]
- **Catechol:** It has a role as genotoxin, an allelochemical and a plant metabolite. They act as a precursor to pesticides, flavors and fragrances.





Catechol **β-sitosterol**

Fig 2.7: Chemical structure of sterols and polyphenols present in *Rheum* species

Structure source: PubChem

A significant amount of research has been conducted to determine the presence of phytochemicals in *Rheum* species. This research has led to the discovery of several classes of phytochemicals, including anthraquinones, stilbenes, flavonoids, acylglucosides, and essential oils. These compounds have been found to have various health-promoting effects, including anti-inflammatory, antioxidant, anti-cancer, anti-bacterial, and anti-fungal properties. The presence of these phytochemicals in *Rheum* species is of particular interest due to their traditional use in Chinese herbal medicine to treat a variety of health conditions.

Numerous analytical methods have been employed by various researchers to isolate anthroquinones in different types of Rheum. These methods include TLC, HPLC, HPTLC, GC-MS, SFC, Microemulsion Electrokinetic Chromatography etc. Thin layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) are the most commonly used techniques for identifying anthraquinone compounds in *Rheum* species. He and Luo (1980)[71] used TLC, while Liu *et al*. (1999)[72] used HPLC for their analysis. In 1999, Rumpunen *et al* studied about the phytochemical and morphological characterization of different cultivars of culinary rhubarb.[73] Different components like total oxalate, malate, anthocyanin, total sugars were analysed and their presence was confirmed by HPLC.

Yadav *et al.*, (2011) investigated the occurrence of phytochemicals using soxhlet apparatus with different alcoholic solvents and determined the total phenolic and flavonoid contents of the several

medicinal plants. [74] The research demonstrated that both crude aqueous and organic solvent extracts encompass valuable bioactive components with medicinal significance. This validates their application in traditional remedies aimed at addressing various ailments. The comparative variation in active constituents of 2 species of *Rheum* Namely *Rheum acuminatum and Rheum austral* was studied by Rokaya *et al.,* (2012).[5] The comparison was done between the rhizomes extract of cultivated and naturally growing plants of both of the species. The research findings indicate that cultivated plants are suitable for obtaining elevated levels of piceatannol and resveratrol, which are stilbenes. Conversely, plants sourced from natural habitats are more suitable for extracting increased quantities of other compounds like anthraquinone. However, they didn't explain any reason for this irregular presence of phytochemicals in different species. Various pharmacognostic and physicochemical measures to authenticate drugs sourced from *R. emodi* was performed by researchers. According to researchers, the phenolic and flavonoid contents present in *Rheum* rhizomes were 0.68% w/w and 0.28% w/w, respectively. Additionally, a HPTLC fingerprint profile of the methanolic extract, validated the occurrence of phenols and flavonoids .[21]

In their study, Wani *et al.,* (2012) examined the methanolic and aqueous extracts of R. emodi using thin layer chromatography (TLC) with a solvent mixture of chloroform and methanol (80:20).[75] The aqueous extract was found to contain more flavonoids and terpenes, as well as small quantities of alkaloids, tannins, and carbohydrates. In contrast, the methanolic extract had high levels of glycosides, flavonoids, terpenes, and saponins, but relatively low levels of alkaloids and tannins. Similarly, the existence of varied groups of phyto-constituents that are responsible for the anti-fungal, anti-microbial, anti-proliferative, immune - enhancing, anti-viral and anti-oxidant activities were studied by Nazir *et al.,* (2013), Manik Sharma *et al*., (2019), *Zhumashova et al.*, (2019), Taskin *et al*., (2019).[20,76,77,78]

Tabin *et al*. (2016) conducted a phytochemical investigation on *Rheum spiciforme and Rheum webbianum,* examining various plant components from both wild populations and tissue culture-grown plants.[35] They utilized HPLC to quantify the presence of anthraquinone derivatives, specifically emodin, aloe-emodin, and rhein. The study supports that the therapeutic properties of plants are due to the phytochemicals present. The role of different phytochemicals present in *Rheum spiciformis Royle* were studied by Bhat *et al*., (2018). The plant extracts were obtained via

soxhlet apparatus using different solvents and then assessed for total phenolic, DPPH anti-oxidant activity, reducing power, hydroxyl radicle scavenging activity.[79] The outcomes unveiled that these secondary metabolites have the potential to function as antioxidants and anti-inflammatory agents, offering a possible defence against diverse pathological conditions linked to oxidative stress.

A recent breakthrough has proposed that utilizing in-silico methods, phyto-compounds like emodin, thymol, and carvacrol have the capability to directly attach to SARS-CoV-2 spike glycoproteins. This implies their potential as powerful candidates for drug exploration against COVID, as suggested by Rolta *et al*. (2021)[80].

The discovery and investigation of phytochemicals is crucial as they offer potential therapeutic benefits and may provide an alternative to conventional medicine. Thus, understanding the presence and usefulness of phytochemicals in plants is essential for developing effective and safe therapies for various health conditions.

2.7 Pharmacological Studies

There have been several pharmacological studies conducted on different species of Rheum. These studies have explored the potential beneficial effects of *Rheum* in various areas like Anti-inflammatory, Anti-oxidant, Anti-microbial, Anti-cancer, Anti-diabetic etc. Overall, these studies put forward that *Rheum* species have beneficial medicinal effects and can be used in the development of new drugs to treat various diseases. The activities are due to certain secondary metabolites and phytochemicals isolated from them.

2.7.1 Anti-Inflammatory Activity

The ability of compounds produced from rhubarb to block pro-inflammatory pathways dependent on nuclear factor kappa B (NF-jB) is thought to be the cause of their anti-inflammatory effects. An aqueous extract from *R. rhabarbarum* was found to suppress the activation of NF-jB-p65, which is implicated in the inflammatory response, when it was triggered by tumour necrosis factor an in a study on human umbilical vein endothelial cells (HUVECs).[81]

2.7.2 Antifungal Activity

Agarwal *et al*., (2000) tested different phytochemicals (Rhein, physcion, aloe-emodin, and chrysophanol) extracted from *Rheum emodi* rhizomes for their antifungal activity against different fungal strains responsible for skin diseases in India (*Candida albicans, Cryptococcus neoformans, Trichophyton mentagrophytes, and Aspergillus fumigatus*). The minimum inhibitory concentration (MIC) was found to be between 25-250 µg/ml[82]. In another experiment Babu *et al*. (2003) used three compounds (revandchinone-1, 3, and 4) extracted from *Rheum emodi* rhizome exhibited antifungal activity against *Rhizopus oryzae and Aspergillus niger,* with MICs of 8–9 and 9–11 mm for the corresponding test concentrations of 100 and 150 g/ml. [83]

2.7.3 Antibacterial Activity

According to research conducted by Ibrahim *et al*., (2006) the extracts (ethanolic and benzene) obtained from *R. australe* have demonstrated strong activity against 30 isolates of *Helicobacter pylori,* which were resistant to other treatments.[84] These isolates were obtained from gastric biopsy specimens. The extracts also exhibited significant activity against gram + bacteria, including *Staphylococcus aureus and Bacillus subtilis,* and gram-negative bacteria, including *Escherichia coli and Proteus vulgaris,* both in vitro and in vivo. In several studies, the rhizome extracts of *R. australe* have demonstrated promising activity against various bacterial strains, including Helicobacter pylori and gram-positive and gram-negative pathogenic bacteria. The extracts have been found to be more effective against certain bacterial species, such as *S. aureus, Enterobacter aerogenes, E. coli, and Citrobacter frundii,* with MIC ranging from 0.16 to 25 µg/ml.[85] Recent research has also shown that the hydromethanolic extract of *R. australe* has antimicrobial activity against acute gastroenteritis bacterial strains, with the lowest minimum inhibitory concentration observed in *S. infantis*.[86]

2.7.4 Antimicrobial Activity

Babu *et al*., (2003) studied the antimicrobial activity of compounds from *Rheum emodi*. The potential of compounds was evaluated against Gram (+) (*Bacillus subtilis*, *Bacillus sphaericus,* and *Staphylococcus aureus)* and Gram (-) bacteria (*Klebsiella aerogenes, Chromobacterium*

violaceum, and *Pseudomonas aeruginosa).* In the tested compounds, revandchinone-4 displayed elevated antibacterial potency, exhibiting inhibition zone diameters of (12-14 mm) at test concentrations of 30 and 100 g/ml. In contrast, revandchinone-1 and revandchinone-3 exhibited lower antibacterial efficacy, with inhibition zone diameter of 7-9 mm at the same test concentrations. [83]

2.7.5 Antioxidant Activity

In the study by Rajkumar *et al.*, (2011), the antioxidant attributes of methanolic and aqueous extracts of *Rheum emodi* rhizome were assessed. This involved scrutinizing their efficacy in scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals, inhibiting lipid peroxidation (LPI), and displaying Fe^{3+} reducing antioxidant properties. The extracts exhibited notable levels of phenolic compounds, which demonstrated a robust positive correlation with their abilities to scavenge free radicals (DPPH and OH), inhibit LPI, and reduce Fe^{3+}.[48]

2.7.6 Anticancer Activity

Rajkumar *et al.*, (2011, b) conducted an experiment to determine the potential cytotoxic effects of the extracts of *Rheum emodi* rhizome on human breast carcinoma (MDAMB-435S) and liver carcinoma (Hep3B) cell lines. According to their findings, the extracts demonstrated significant conc-dependent cytotoxicity in the tested cells. [87]

2.7.7 Antiulcer Activity

In a study conducted on rats with pyloric ligation-induced ulcers, Amandeep et al. (2012) investigated the antiulcer potential of the ethanolic extract of *Rheum emodi* rhizome. The findings revealed a lowered ulcer index, reduced volume and total acidity of gastric fluid, and an elevated pH. [194]

2.7.8 Antidiabetic Effect

Radhika *et al.*, (2010) studied the potential antidiabetic effects of *Rheum emodi* rhizome extract and its impact on glucose metabolism enzymes in normal and diabetic rats. A 75% ethanolic extract (250 mg/kg) reduced certain enzyme activities in liver and kidney tissues while enhancing others[89]. Similarly, in a different study, Radhika *et al.*, (2012) examined the therapeutic effects of the same

extract on biochemical markers in serum, liver, and kidney tissues of diabetic rats. Administration of 250 mg/kg extract for 30 days restored marker enzymes to nearly normal levels. [90]

2.7.9 Antiplatelet and Anticoagulant Activities

Seo EJ *et al.*, (2012) conducted a study to explore the impacts of anthraquinone derivatives extracted from rhubarb on platelet activity. Among the four tested anthraquinone derivatives, chrysophanol-8-O-glucoside (CP-8-O-glc) demonstrated the highest efficacy as an inhibitor of collagen- and thrombin-induced platelet aggregation. The administration of CP-8-O-glc led to considerably extended bleeding times in mice. Furthermore, CP-8-O-glc displayed a significant inhibitory effect on ex vivo rat platelet aggregation and on thromboxane A2 formation in vitro. [18]

2.7.10 Antidyslipidemic Activity

Mishra *et al.*, (2013) showed the antidyslipidemic effects of an ethanolic extract of *Rheum emodi.* The active constituents of the extract were identified as chrysophanol, emodin and emodin 8-O-β-D glucopyranoside. The study found that these constituents had a significant effect in reducing plasma lipid levels in triton-induced rats. Emodin was found to have a lipid-lowering activity in a high-fat diet model. [91]

2.7.11 Immune-Enhancing Effect

In their study, Kausar F *et al.* (2011) explored the immune-enhancing potential of the ethyl acetate rhizome extract of *Rheum emodi* on cell lines. The extract was able to improve the immune system by inducing the release of various cytokines. The study showed that there was a growth in the release of Nitric Oxide (NO) and cytokines TNF-α, IL-12, in a dose-dependent manner, and a reduction in IL-10 by RAW 264.7 macrophages cell line when treated with the extract alone. [93]

2.7.12 Hepatoprotective Activity

Ibrahim *et al.*, (2008) conducted a study to evaluate the hepatoprotective activity of the ethanolic extracts of *Rheum emodi* rhizome against CCl4-induced liver damage in Wistar rats. The introduction of carbon tetrachloride led to a notable rise in the serum levels of AST, ALT, ALP, and total bilirubin. Nonetheless, oral administration of *Rheum emodi* at a dose of 3.0 g/kg demonstrated significant reduction in these escalated indicators and reinstated the standard liver

architecture[93]. Similarly, Akhtar *et al*. (2009) corroborated the hepatoprotective properties of the aqueous extract of *Rheum emodi* against paracetamol-induced liver damage in albino rats. [94]

2.7.13 Nephro-Protective Activity

Alam *et al*. (2005) explored the impacts of the water-soluble (W-S) and water-insoluble (W-INS) components within the alcoholic extract of *Rheum emodi* on nephrotoxicity triggered by $CdCl_2$, $HgCl_2$, $K_2Cr_2O_7$ and gentamicin in both rats with induced nephrotoxicity and normal rats. The study focused on assessing serum urea nitrogen and creatinine levels. The results revealed that the W-S fraction exhibited a nephron-protective influence on all segments of the proximal tubule (S1, S2, and S3), likely attributed to the anti-oxidative properties of the tannins present in the fraction. [95]

2.7.14 Cardio-protective Properties

The cardio-protective properties of phyto-based products are primarily due to their anti-oxidant and anti-inflammatory activities. A recent study on the ability of compounds found in *R. rhabarbarum* to inhibit a-glucosidase, an enzyme involved in carbohydrate metabolism, produced varied results. While most of the compounds tested had IC50 values exceeding 100 lM, two compounds, d-viniferin and rhapontigenin, showed higher inhibitory activity with IC50 values of 0.5 lM and 15.4 lM, respectively, compared to acarbose, a commonly used inhibitor of a-glucosidase, with an IC50 value of 126.8 lM.[96] According to a study by Yoo *et al*. in 2007, when testing seven compounds of stilbene type found in Rhubarb rhizome on isolated rat aorta, it was found that piceatannol had the strongest vasorelaxation effect with an EC50 value of 2.4 lM. [97]

2.7.15 Menopausal Complaints

Rheum species contains phytochemicals that have estrogenic properties, which has led to its recognition as a probable source of remedy for hormone-related disorders, including menopausal symptoms. Several studies, including those by Chang *et al.* (2016),[98] Hasper *et al*. (2009),[99] and Heger *et al*. (2006),[100] have investigated the medicinal potential of this plant. The estrogenic effects of *R. rhaponticum* are mainly due to the presence of hydroxystilbene compounds like rhaponticin, resveratrol, and piceatannol etc.

Any concentrates of *Rheum emodi* or any other species should not be used by individuals with gout, rheumatism, epilepsy etc. Due to the rhizome's oxalic acid content, which can react with bloodstream calcium to produce insoluble calcium oxalate crystals that could end up in the kidneys or gall bladder. It is not recommended for use in cases of inflammation or fever, although it may sometimes cause irritation. Additionally, internal use of the roots can cause urine to have a deep colour.[49]

2.8 Genetic Diversity Assessment

Biological diversity encompasses the diversity of species, their genetic variations, and the complex ecosystems that they form. It plays a critical role in sustaining the balance of the Earth's climate and mitigating the impacts of climate change. Unfortunately, biodiversity is currently under threat due to human activities like habitat destruction, overexploitation of natural resources, pollution, and climate change. This harm of biodiversity has extensive consequences for the planet, including the extinction of species, disruption of ecosystems, and loss of important ecosystem amenities that are essential for human well-being. There are three main types of biodiversity:[101]

1. Genetic diversity: This pertains to the range of genes and genetic variances present within a population or species. Genetic diversity holds significance as it enables adjustments to evolving environmental circumstances and enhances the capacity of populations to withstand diseases and other potential risks.

2. Species diversity: This denotes the assortment of distinct species present in a specific ecosystem or locality. Species diversity carries significance as it contributes to the overall well-being and stability of the ecosystem, and offers vital ecological functions like pollination and nutrient cycling.

3. Ecosystem diversity: This encompasses different ecosystems that exist on Earth, from forests and grasslands to wetlands and coral reefs. Ecosystem diversity is important because it supports a wide range of species and provides important ecological services such as carbon sequestration, water filtration, and soil formation.

One more type of diversity is genomic diversity that covers the diversity at several gene loci across the genome.[102] All these types of biodiversity are interconnected and contribute to the overall

health and resilience of the planet. Loss of any one type of biodiversity can have cascading effects on the entire ecosystem, and ultimately on human well-being.

Human interventions like habitat degradation, pollution, excessive utilization of natural resources, and alterations in climate, can have significant impacts on genetic diversity. When natural habitats are destroyed or fragmented, populations of species can become isolated and their genetic diversity can be reduced. This can increase the risk of inbreeding, decrease the capability of populations to adapt to changing environmental conditions, and increase the risk of extinction. Human interventions are reducing GD in many populations, which can have negative impacts on their ability to adapt to changing environmental conditions and survive over the long term. Therefore, the sustainable use of biological diversity is of critical importance.[103]

Genetic diversity plays a key role in safeguarding the viability of plant species within natural ecosystems and advancing crop enhancement efforts. The expansive array of genetic resources present in plants equips breeders to create novel and enhanced cultivars possessing desirable traits. These attributes encompass those favoured by farmers, like elevated yield potential and substantial seed size, alongside those favoured by breeders, such as resistance to pests and diseases, and sensitivity to light. Consequently, the genetic diversity inherent in plant resources empowers breeders to forge new cultivars that better suit shifting environmental circumstances, exhibit heightened performance, and exhibit enhanced resilience.[102] Overall, genetic diversity serves as a valuable asset for breeders, empowering them to generate fresh, improved cultivars adept at navigating evolving environmental conditions and societal demands.

2.9 Methods of Genetic Diversity Analysis

Diversity analysis can be achieved through certain approaches, including morphological, cytological, biochemical, and molecular characterization. Initially, diversity analysis relied on morphological markers, which represented inherent variations within a given plant species. Subsequently, distinctions at the cytological and biochemical levels were also harnessed for assessing GD. As genomic tools advanced, molecular markers have emerged as the preferred technique for assessing GD. These markers provide more precise and accurate information about genetic diversity and can be used to analyse a large number of genotypes. Overall, diversity

analysis can be carried out using different methods, with molecular markers being the preferred approach for assessing GD due to their accuracy and high throughput capacity.

2.9.1 Morphological Markers

Morphological characterization involves evaluating the physical characteristics of different plant entries that are grown in the field. These characteristics are the most important factors in determining the agronomic significance and the taxonomic categorization of plants. Morphological assessments are cost-effective, simple to perform, and they don't necessitate sophisticated technology. By observing and measuring the physical traits of plants, researchers can gain insight into their genetic makeup, growth patterns, and overall health. This information is essential for plant breeding programs and for conservation efforts aimed at preserving genetic diversity in plant populations. [104,105]

2.9.2 Cytological Markers

Cytological features encompass the examination of diverse attributes of chromosomes viz. dimensions, secondary constrictions, centromere placement, arm proportions, constitutive heterochromatin patterns, and banding traits (G, Q, R, and N banding). Additional factors involve DNA content, total genomic chromosome length, and chromosome volume. These characteristics have been employed to evaluate GD both within and among species in crops like maize, potato, lentil, radish, and tomato. Nonetheless, the utility of these features in genetic diversity analysis is confined by their scarcity and limited resolution. While cytological features provide important information about genetic diversity, they are not as precise or comprehensive as molecular markers, which are now widely used for genetic diversity analysis. [106,107]

2.9.3 Biochemical Markers

Biochemical analysis involves the separation of proteins (isozymes) into distinct banding patterns. These isozymes represent outcomes of diverse alleles, rather than the genes directly. By mapping these isozymes onto chromosomes, they can serve as genetic markers for mapping other genes. This technique offers an instant approach for evaluating GD and necessitates a smaller plant tissue sample. However, the number of possible isozymes is limited, and changes in the environment can affect how an isozyme expresses itself. Isozymes also possess limitations, as they cannot be utilized to construct an all-encompassing genetic map; they only offer insights into a fraction of the genome. Despite these limitations, biochemical analysis remains a useful tool for assessing

genetic diversity, especially in situations where other methods, such as molecular markers, are not available or feasible. [108,109,110]

2.9.4 Molecular Markers

Molecular characterization encompasses the examination of variations among genotypes at the DNA/RNA level. Various molecular markers possess distinct attributes that render them appropriate for diverse objectives. Different techniques of molecular assessment can evaluate multi-locus or single-locus markers. Multi-locus markers use oligonucleic primers with arbitrary sequences to amplify casual chromosomic traits of several genomic loci simultaneously. These markers are dominant, as they can only determine the presence or absence of a band for any locus, without distinguishing between heterozygote (a/-) and homozygote (a/a) conditions for the same allele. Conversely, single-locus markers utilize probes or primers that are targeted to specific genomic loci. These markers can hybridize or amplify chromosome features possessing clearly defined sequences. They exhibit co-dominance, enabling differentiation between homozygous and heterozygous loci.

Molecular markers are highly informative, and their high level of polymorphism allows for accurate assessment of genetic diversity. They are also highly reproducible and can be used to make a genetic map. However, molecular markers are relatively expensive, require specialized equipment and expertise, and can be time-consuming to develop and analyse.

According to Mondini *et al*., 2009 a desirable molecular marker should be polymorphic in nature, uniformly dispersed across the genome, should be capable of producing dependable markers that distinctly highlight genetic disparities, straightforward, rapid, and cost-effective, demands only minimal quantities of tissue and DNA samples and should be associated with distinct phenotypes without necessitate prior knowledge about the organism's genome. [108]

Molecular markers can be classified as hybridization-based or PCR-based. Hybridization-based markers, such as RFLP and AFLP, involve hybridizing a labelled probe to DNA fragments of interest, while PCR-based markers, such as RAPD, ISSR, SSR and SNP, involve amplifying specific DNA regions using PCR) and detecting sequence variations.

2.9.4.1 Non-PCR-Based Techniques

In the early stages of plant study, restriction-hybridization-based molecular markers were used. Hybridization and the use of restriction endonucleases were engaged in this. Molecular markers such as RFLP and VNTRs are examples of restriction-hybridization-based techniques. By hybridizing a tagged DNA probe to a Southern blot containing DNA that has been digested by restriction endonucleases, RFLP markers can identify DNA polymorphism. The result is a characteristic DNA fragment pattern. These markers enable the simultaneous screening of several samples and are highly polymorphic, codominant, repeatable, and reproducible. However, this technique is not widely used due to its time-consuming nature, requirement for large quantities of high-quality genomic DNA, and the use of expensive and potentially hazardous radioactive reagents. Additionally, previous sequence information is required for probe generation, which further complicates the methodology. As a result, PCR-based techniques were developed as a less complex alternative.[33]

2.9.4.2 Markers Based on Amplification Techniques (PCR-Derived)

The development of Polymerase Chain Reaction (PCR) by Mullis *et al*., 1986 has greatly facilitated the usage of molecular markers, particularly the PCR-based techniques.[111] This method amplifies distinct DNA products from segments of DNA that are bordered by regions exhibiting significant similarity to the primers. There are two subdivisions of PCR-based techniques:

(1) Arbitrarily primed PCR-based techniques

(2) Sequence targeted PCR-based techniques

I. Random Amplified Polymorphic DNA (RAPD)

RAPD (Random Amplified Polymorphic DNA) was introduced by Welsh and McClelland in 1990.[27] RAPD markers are generated through the random amplification of genomic DNA using brief primers known as decamers.'The amplified fragments are separated on an agarose gel with the help of EtBr, and the resulting fragments are visualized under ultraviolet light. Using short primers is important because it increases the likelihood that even random sequences can find homologous sequences that can anneal. DNA polymorphisms are created by rearrangements or

deletions at or between oligonucleotide primer binding sites in the genome. The advantage of using RAPD markers is that it does not require existing knowledge of the genetic material. Since RAPD markers are dominant, it cannot distinguish between homozygous and heterozygous loci. However, they are reproducible, simple, inexpensive, and require only a small amount of DNA sample (around 100 ng). [112,108] Recently RAPD is used to access the GD in Onions,[113] Saffron,[114] Rice[115] and several other plant species. RAPD is widely used to evaluate the GD and genetic mapping in several endangered herbs of Indian Himalayan region. [116,117,118]

II. Amplified Fragment Length Polymorphism (AFLP)

AFLPs are generated through a combination of restriction endonuclease digestion and PCR amplification, as first described by Vos *et al*. in 1995.[26] This technique uses a combination of rare cutter and frequent cutter specific enzymes for the digestion step. AFLP markers are considered an intermediate marker between RAPD and RFLP due to their higher reproducibility and sensitivity compared to RAPD, and their ability to analyse more loci than RFLP. AFLP markers typically produce a range of 50-100 bands per assay, and the no. of amplified fragments is determined by various factors such as the number of selective nucleotides in the AFLP primer combination, GC content, genome size etc. AFLP markers can generate unique patterns of any DNA sample, irrespective of the organism's origin or the DNA sequence. Since most AFLP fragments represent distinct regions on the genome, they can serve as genetic and physical markers. AFLP is capable of discriminating closely related individuals within a subspecies and is also useful for gene mapping.[119] For the determination of the relationship between the phenotypic characteristics and GD, AFLP markers are widely used. AFLP analysis has been widely used AFLP markers have been used to provide an estimate of Rhubarb GD. Joseph et al., 2008 generated the fingerprint analysis of 37 cultivars and 4 accessions of *Rheum* species. They analysed the 10 Eco RI and MseI primer combinations, for 1400 scored polymorphisms. The results displayed at least two clusters of related cultivars with distantly related accessions. [120] Thereby providing an estimate of rhubarb cultivar genetic diversity.

AFLP can also be applied in gene mapping, DNA Fingerprinting[121,122] and genetic diversity assessment. [123,124,125]

III. Inter-Simple Sequence Repeats (ISSR)

ISSR markers can be used to characterize GD (Genetic Diversity) by generating DNA fingerprints of individuals or populations based on the unique banding patterns produced by the amplification of these regions. The ISSR technique has several advantages, such as the ability to amplify DNA from a broad range of taxa, the reproducibility of the results, and the ability to generate many polymorphic bands in a single reaction. To use ISSR markers for genetic diversity studies, researchers design primers that target regions with high variability and PCR amplify these regions using genomic DNA from individuals or populations of interest. The resulting PCR products are then electrophoresed and visualized using gel staining or fluorescent dyes. The unique banding patterns can then be analysed to assess GD, population structure, gene flow, and other parameters. ISSR markers have been used for the assessment of GD in Saffron[114], Onions[113], Safflower[127] and other medicinal plant species.[116]

IV. Sequence Specific PCR Based Markers

An alternative to PCR amplification is to amplify distinct sections of a genome by utilizing targeted primers. Recent advancements in high-throughput sequencing technology have yielded abundant data on DNA sequences for numerous plant species' genomes. Moreover, Expressed Sequence Tags (ESTs) have been generated for many crop species, with thousands of sequences annotated as potential functional genes using powerful bioinformatics tools. EST libraries offer a glimpse into the genes that were active in the tissue at the specific time and under the particular conditions during which they were collected. In contrast to SSRs, EST-SSRs are a rapid, efficient, and cost-effective approach.[128] The EST-SSR technique has been used to analyse the GD of cultivated and elite barleys.[122] Additionally, data from EST libraries can be utilized to develop tools such as quantitative PCR assays and microarrays that can identify gene expressions in species.[108]

V. Microsatellite-Based Marker Technique

VNTRs are specific sites on DNA where a short sequence of nucleotides, known as Simple Sequence Repeats (SSR) or Short Tandem Repeats (STR), are repeated in tandem. These repetitive regions are also called microsatellites. Microsatellites offer several advantages such as high abundance, vast genetic diversity, co-dominant inheritance, reproducibility, and the ability to detect length variations using flanking primers. Recent technological advancements have shifted microsatellite analysis from hybridization-based approaches to PCR-based approaches. The

fundamental valuation methods developed from microsatellite analysis include SCARs (Sequence Characterized Amplified Region), STMSs (Sequence Tagged Microsatellite Site), CAPS (Cleaved Amplified Polymorphic Sequences), SSLPs (Simple Sequence Length Polymorphism) and SNPs (Single Nucleotide Polymorphisms).[108]

The SSR or microsatellites are applied in genome mapping, evolutionary studies, genotyping genetic diversity analysis etc.[129,130,124]

2.9.4.3 DNA sequence-based Techniques

DNA is composed of four Nucleotide bases: Adenine, Guanine, Thymine, and Cytosine. DNA sequencing involves the process of deciphering the arrangement of these nucleotide bases within a DNA molecule. Franca *et al.,* 2002 focused on the four most well-known DNA sequencing techniques and practical considerations such as read-length, speed, accuracy, throughput, cost, and sample handling automation. [131] The methods discussed are:

(i) Sanger method and its various enzymatic variants;
(ii) Maxam & Gilbert method (Chemical methods);
(iii) Pyrosequencing, which enables real-time DNA sequencing by detecting released pyrophosphate;
(iv) Single molecule sequencing employs exonuclease digestion of an individual molecule comprised of a solitary strand of deoxy-nucleotides that are labelled with fluorescence.

Overall there are two types of DNA sequencing techniques: Conventional sequencing and Next-generation sequencing (NGS). Examples of DNA sequence-based techniques include TGGE, DGGE, CAPS, SSCP, STMS, and HETERODUPLEX.[30]

2.10 Characterization of Genetic Diversity using RAPD markers

Random amplified polymorphic DNA (RAPD) markers are widely used to characterize GD within and among populations of various organisms. RAPDs are based on the PCR amplification of random genomic DNA segments using short, arbitrary primers that bind to multiple sites in the genome. RAPD markers are advantageous because they are easy to use, do not necessitate prior familiarity with DNA sequences, and can produce many polymorphic bands in a single PCR reaction. RAPDs have been used to study genetic diversity, population structure, gene flow,

hybridization, and phylogenetic relationships in a varied range of organisms (plants, animals, fungi, and bacteria).

Rheum species are endangered due to overharvesting and habitat destruction. Genetic diversity analysis can help identify genetically diverse populations that should be prioritized for conservation efforts. A lot of studies have been done so far employing the use of RAPD markers for evaluating the GD in *rheum* species.

The molecular-level diversity in *Rheum L*., were conducted by Yang *et al*., 1994 using a random amplified polymorphic DNA (RAPD) analysis.[132] The method displayed to be dependable, precise, efficient, and consistent in verifying the authenticity of official and unofficial plants of *Rheum L*. Furthermore, the findings of the study suggest that the use of bulked segregate analysis (BSA) is a more efficient method for screening primers compared to using individual selection. In 2000 Persson *et al.*, used 47 different RAPD (Random Amplified Polymorphic DNA) markers to discriminate the Genetic Diversity (GD) among twelve culinary Rhubarb (*Rheum* spp.) cultivars. Considerable variability was found out among the species giving data for diversity analysis.[133]

Moon *et al.* (2009) established a dependable approach to accurately identify and enhance the quality standards of *Rhei Radix et Rhizoma* and *Rhei Undulatai Rhizoma*.[134] This was achieved through the analysis of RAPD (Random Amplified Polymorphic DNA) and the subsequent development of a SCAR (Sequence Characterized Amplified Region) marker. This marker proved efficient in distinguishing between plant species and commercially used herbal medicines. RAPD markers are also used to develop DNA barcoding of medicinal plants for identification purpose. The genetic diversity in nine accessions of *R. officinale* using RAPD markers was assessed by Subositi *et al*., in 2022. The genetic similarity among the populations was found out to be 74.58 % providing information for future conservation and management purposes.[135]

However, RAPD markers have some limitations, including their susceptibility to technical variability, lack of reproducibility, and inability to detect variation in certain regions of the genome. Therefore, RAPDs are often used in combination with other molecular markers, such as microsatellites or SNP markers, to provide a broader view of GD.

2.11 Characterization of Genetic Diversity using ISSR markers

Inter-Simple Sequence Repeat (ISSR) markers are molecular markers that use primers that bind to microsatellite-like regions of DNA. ISSR markers are based on PCR amplification of the DNA among two microsatellite regions using primers that target these regions, resulting in the amplification of a variable region between them. Genetic diversity analysis can be used to identify genetically diverse individuals or populations that can be used in breeding programs to improve the yield and quality of *Rheum* plants.

Wang *et al.* (2012) used ISSR markers to study the GD within and between populations of two *Rheum* species, *R. tanguticum and R. palmatum*.[29] They selected 30 populations of the two species, covering the entire distribution range in China, and analyzed a total of 574 individuals using 12 ISSR primers. The findings indicated that the majority of fragments exhibited polymorphism, underscoring a notable extent of genetic diversity. The GD between *R. palmatum and R. tanguticum* was relatively minor, implying a closely linked genetic affiliation between these two species. The study highlights the potential of ISSR markers for characterizing the genetic diversity of *Rheum* species and understanding their evolutionary relationships.

The genetic variety and structure of three *Rheum* species, *R. emodi, R. webbianum, and R. spiciforme,* were investigated using ISSR markers in the Kashmir region. The study analysed 45 individuals from the three species and found high relevance among individual species with high genetic variations. The Nei's gene diversity was calculated and showed that *Rheum webbianum* had the highest GD with a value of 0.345, followed by *R. spiciforme* with 0.241, and *R. emodi* with 0.237. However, some populations of *R. emodi* displayed less genetic diversity, indicating the loss of genetic material in the future if no preventive steps are taken. Furthermore, ISSR markers were used to evaluate the degree of genetic organization and variation within five cultivated populations of *R. tanguticum*. [25,7,136]

Yanping Hu *et al.*, 2014 analyzed the levels of genetic variations in different populations of *R. tanguticum* employing the use of ISSR markers and illustrated that the Nei's gene diversity was lower in cultivated populations as compared to wild populations.[137] Likewise, in 2014, Gilmore and colleagues assessed the genetic variability of culinary rhubarb (*Rheum rhabarbarum*) cultivars through morphological traits. They also created 96 novel SSR primers, of which the 25

(polymorphic and easily identifiable primer pairs) were successfully employed in the fingerprinting of rhubarb cultivar.[138]

The genetic assessment of *Rheum ribes L*. (wild rhubarb) in Turkey using SSR and ISSR markers indicated that the SSR markers showed a 100% polymorphism rate, while the ISSR markers had a lower polymorphism rate. However, the ISSR markers had a higher average Polymorphism Information Content (PIC) value of 0.805 compared to the SSR markers, which had an average PIC value of 0.724.[139] ISSR markers can be employed alongside other molecular markers like RAPDs or microsatellites to offer a universal perspective on genetic variation. Other than ISSR and RAPD markers SSR have been reported to be used for genetic diversity analysis.

The genetic variation among different populations of *R. tanguticum* in China has been depicted by analyzing chloroplast DNA mat K sequences. The outcomes of AMOVA and PERMUT analyses showed an average genetic diversity within the species of 0.894. Notably, the genetic variation among populations was comparatively higher (67.6%) compared to the variation within populations (13.88%).[137] Fangjian *et al.,* 2014 studied the plant species *Rheum tanguticum*, which is native to the Qinghai-Tibetan Plateau and considered endangered using seven pairs of Simple Sequence Repeats (SSR) markers.[23] The research examined 114 individuals spanning across 10 distinct populations and identified a total of 102 alleles. The average count of alleles per locus stood at 14.6, with expected heterozygosity ranging from 0.384 to 0.515, averaging at 0.459. The genetic differentiation between these populations was notably high (Fst = 0.249), while gene flow remained restricted (Nm = 0.754). This indicated that approximately 21.18% of genetic variations were observed between populations.[23] The study proposes that the species' vulnerable status is likely attributed to human activities like uncontrolled harvesting, rather than a deficiency in genetic diversity.

There are no reports available on the genetic assessment analysis of *Rheum* species in the Uttarakhand region of India. Furthermore, only a few studies had been conducted on the genetic diversity of *Rheum* species in India as a whole. This lack of research on the GD of *Rheum* species in the region highlights a significant gap in our understanding of the diversity and possible uses of these species. Further research in this area could help to inform conservation efforts and improve our knowledge of the medicinal properties of these plants.

Limited genetic diversity information is available for Rheum sp. due to the small number of samples studied. This lack of genetic information can have negative consequences for the conservation of vulnerable species. Hence, there is a crucial need to amass additional genetic data concerning *Rheum* species. Biotechnology has potential applications in improving medicinal plant species, and although it is sometimes used as a supplement or alternative to traditional breeding programs, there is still a significant gap between traditional and biotechnological methods. DNA analysis is a superior option for genetic markers compared to other methods.

2.12 Threat Status and Conservational Measures

According to the International Union for Conservation of Nature and Natural Resources (IUCN), 1 out of 10 species of tracheophytes i.e. vascular plants on earth are vulnerable due to their overuse in commercial and industrial applications. One such species is *Rheum* species, a major medicinal plant widely used in the drug industry due to their medicinal uses. The excessive exploitation of the plant has led to its depletion and it is currently facing immense pressure to survive in its natural habitat. The species is considered critically endangered according to IUCN criteria, and its status has changed from threatened to endangered. The National Medicinal Plants Board has recommended immediate attention for its conservation and cultivation. The plant possesses anti-cancer, anti-bacterial, anti-fungal, and anti-ulcer properties, making it essential in Ayurvedic and Unani medicine. Overexploitation, grazing, uncontrolled deforestation, selective extraction, rapid industrialization, and other factors have contributed to its threatened status in the Northern region of India, particularly in Garhwal Himalayas. Thus, urgent measures are needed for its conservation and cultivation.[140]

Kameshwara Rao (2004) highlighted the significance of biotechnological approaches as novel pathways for safeguarding and exploiting genetic resources.[193] Methods such as in vitro cultivation and cryopreservation have enabled the protection of genetic resources, especially for species that pose challenges in seed preservation. Moreover, progressions in technologies like ELISA and PCR have enabled more precise and pathogen-targeted assessment of seed health. Tissue culture methods are also commonly used to eliminate systemic diseases and ensure the secure transfer of germplasm. These technological developments (viz. molecular markers) are broadening the possibilities for the utilization of genetic resources. “Modern Biotechnology” has expanded the range of ex situ propagation and preservation methods, which were traditionally used for

horticultural and agricultural species, to include endangered and rare species and plant germplasm. Rheum species, being endangered, requires conservation measures. There are various approaches for plant conservation such as Micro propagation, Protoplast Culture, Somatic Embryogenesis, Micro grafting, and Cryopreservation. Synthetic seed techniques such as cryopreservation and synthetic seed have made it easier to conserve germplasm for a longer time. Many tissue culture laboratories have been established for the micro- propagation of plants, which face difficulties in propagation through conventional horticulture techniques.[141]