# CHAPTER – I
# INTRODUCTION

## 1.1. General Introduction About Plants

The beginning of life on Earth remains a perplexing enigma, as it occurred around 3.8 billion years ago and the Earth itself is over 4.5 billion years old. Despite this, the remarkable properties of medicinal plant species are driving force in the evolution of traditional plant based medicinal practices. Humans have long been fascinated by medicinal plants and have utilized them for various health benefits throughout history (Van WyK and Wink, 2018). For centuries, natural substances from plants, animals and minerals have been the primary source of medicine for treating human illnesses. Remarkably plants have played a crucial role by providing vital therapeutic properties through traditional herbal medicines (Lazaris, 2020).

Traditional medicine, relying on the direct application of medicinal plants, remained the primary sources for treatment until the $18^{th}$ century. Despite having a good knowledge of the effects of these plants on the humans, the active compounds which were involved in treatment remained a mystery. During this time, the Canon of Medicine written by Persian physician Avicenna (Ibn Sina) was widely used as a reference for identifying bioactive compounds in plants. With the advancement of modern science, especially in chemical analysis and the development of instruments such as microscopes, active bioactive compounds were isolated from medicinal plants. This paved the way for the synthesis of these compounds in the laboratory to produce medicine for therapeutic use. Today, medicinal plants play a crucial role in the pharma industries as a source of raw materials for active bio-constituents. However, all the regions of the world may not rely on synthetic medicine. Yet underdeveloped countries, traditional medicinal practices are still demanded due to its affordability (Salmerón and Manzano, 2020).

Ethno-medicine is described as the collective information, aids, and applications based on beliefs and experiences inherent of different regions that are utilized for maintaining health, and preventing, diagnosing, improving or treating physical and mental illnesses, regardless of whether they are explicable or not (WHO, 2000; 2014-2023). It has been widely used due to its natural origins and comparatively low risk of complications. Still the traditional herbal medicine is significant part of history across the world (Süntar, 2020). Plants are a huge manufacturing unit of secondary metabolites, which forms the foundation for many pharmaceutical drugs and

herbal remedies. The chemical compounds present in medicinal plants have health-promoting biological activities (Li *et al*. 2020).

In the world, there are over two lakhs fifty thousand plants, with approximately 80,000 being therapeutic value. India is the richest plant biodiversity with over 45,000 species are found within its various climatic zones. Above 20,000 plants are believed to have medicinal properties, although only 7,000 to 7,500 species are folk medicine. In India, there are various traditional systems of medicine, such as Ayurveda, Siddha and Unani practised since many centuries. The drugs were prepared from various parts of the plants, such as leaves, stems, bark, roots, flowers, seeds and secondary products like gum, resin, and latex (Roy *et al*. 2018).

**1.2. Plant Tissue Culture (PTC)**

It is a method of growing plant cells, tissues or organs under controlled conditions. It is widely used technique in plant breeding, genetic engineering and biotechnology. The process of this method involves the use of explants, such as stem, leaves or root, that are grown in a laboratory under sterile conditions on a nutrient-rich medium. This medium provides the necessary nutrients, hormones and growth factors that are needed for the plant tissue to regenerate into a new plant (Neumann *et al*. 2020).

The history of PTC begins early 1900s, while researchers first began experimenting with growing plant cells in culture. However, it was not until the 1960s and 1970s that significant advances were made in the field, allowing for the successful growth and develop a whole plant from an explant. Since then, it has become an essential tool in breeding of plants and to produce genetically modified crops (Thorpe, 2007).

One of the most significant applications of PTC is in the micropropagation of plants. Micropropagation is the process of producing many genetically identical plants from a small piece of tissue. This technique is especially useful for propagation of rare and endangered plant species, as well as for the commercial production of ornamental plants and fruits. In recent years, many researchers have been working on developing new micropropagation techniques that are more efficient and cost-effective (Cardoso *et al*. 2018; Gupta *et al*. 2020). Another important application of PTC is the transgenic plants, which are genetically modified and express a specific trait which resistance to pests or disease. It is used to introduce new genes into plant cells, which are express the desired. Transgenic plants have been used to improve crop yields and to make crops more resistant to environmental stresses (Vitta kavya *et al*. 2021).

PTC is also used for production of specialized biochemical products such as alkaloids, flavonoids, phenolic components, and terpenoids. These compounds are used in pharmaceutical and food industries. Betul (2020), provided an overview of the various applications of PTC in the production of these bioactive compounds. Increasing interest in the plant tissue culture for the conservation of endangered plants, tropical forests, wetlands, and deserts species. Loyola-Vargas and Ochoa-Alejo (2018) discussed the various techniques employed for conservation plants through PTC. It has become an essential tool in many areas of plant science, including plant breeding and genetic engineering, enhancement of secondary metabolites and conservation of endangered plant species.

**1.3. Phytochemistry**

Plants are a rich assortment of phytoconstituents with greater medicinal values and investigated for various ailments as an alternative to the existing commercial drugs. Phytochemistry is the study of the chemical compounds found in plants, including the processes of their formation, extraction, isolation, identification, and investigation of their biological properties. It also encompasses the study of phytochemicals, which are the chemical compounds found in plants. Plants are considered a rich in various bioactive compounds that possess an extensive therapeutic property. The bio-active compounds present in different parts of medicinal plants, can be used to treat a variety of illnesses and conditions. They are considered an important source of natural medicines and play a crucial role in traditional and modern medicine (Aye *et al*. 2019).

Significance of drug development from natural products has been rising, with plants serving not only as direct sources of bioactive compounds for therapeutic purposes but also as raw materials for drug synthesis or as models for the creation of new biologically active compounds. However, to effectively utilize plants as a source of phytopharmaceuticals, it is essential to conduct extensive research in both basic and applied domains, thereby elevating the importance of this resource to the level of conventional pharmaceutical products (Andrade *et al*. 2018).

Herbal drugs are developed through a combination of scientific research on the bioactive constituents of plants, as well as indigenous knowledge and traditional use of medicinal herbs. This approach to drug development combines both modern and traditional methods to bring herbal remedies to the forefront of healthcare (Oladeji *et al*. 2019). As compared to synthetic

compounds, natural products more closely resemble endogenous metabolites and biosynthetic intermediate active transporters (Heinrich *et al*. 2021).

**1.4. Antimicrobial Activity**

Bioactive compounds of the medicinal plants have potential antimicrobial properties. These plants are used in various forms, for instance teas, decoctions, tinctures, and essential oils have been traditionally used to combat bacterial, fungal and viral infections. The use of these secondary metabolites as antimicrobial agents from plants has been widely studied and has shown promising activity. In addition, the breakthrough of new phytoconstituents from plants continues to broaden the range of natural antimicrobial agents available for use in medicine (Bazzaz *et al.* 2018).

Bioactive compounds have the capacity to inhibit the growth of various microorganisms such as bacteria, fungi, viruses and protozoa. Secondary metabolites employed different mechanisms of action than those of conventional antimicrobial agents, making them potentially useful in the treatment of antibiotic-resistant microbial strains. This significant clinical value as the rise of antibiotic-resistant strains of microorganisms has become major public health concern. Antimicrobial compounds from medicinal plants may provide an alternative treatment for these resistant strains (Vaou *et al*. 2021).

Some of the bioactive compounds found in medicinal plants possess both intrinsic antibacterial activity and the ability to modify microbial infestation. Furthermore, these compounds are often chemically complex, which means they have a lower risk of causing side effects compared to synthetic drugs and a lower likelihood of bacteria developing resistance to them. This makes them highly valuable as potential therapeutics (Ruddaraju *et al*. 2020).

**1.5. Antioxidant Activity**

Reactive Oxygen Species (ROS) is defined as the body has its own internal defence mechanism against the harmful effects of free radicals, which are produced during cellular metabolism. However, the excessive levels of these free radicals can lead to oxidative damage to lipids, nucleic acids, proteins and chronic diseases. To combat these effects, the use of exogenous antioxidants is recommended. These antioxidants can come from either natural or synthetic sources, but the use of synthetic antioxidants is being phased out in favour of natural antioxidants, due to their lower toxicity and cancerogenic properties (Akhtar & Mirza, 2018).

Oxidative stress caused by free radicals and has been linked to the development of several chronic and degenerative diseases. These include conditions such as atherosclerosis, ischemic heart disease, aging, diabetes mellitus, cancer, immunosuppression and neurodegenerative diseases. Bio-active compounds neutralize the free radicals and inhibit the damage caused by oxidative stress (Souri *et al*. 2022). Food industry utilize plant extracts, gained popularity due to their antioxidant properties and potential health benefits for humans. Nowadays people aware of the importance of antioxidant in their diet, and plant-based food is increasing continuously (Tajner-Czopek *et al*. 2020).

**1.6.Cytotoxicity Activity**

Cancer remains to be one of the foremost deadliest diseases worldwide, statistically proven by approximately 8.8 million deaths reported in 2015. According to the WHO, generally these deaths occur in developing countries, with a staggering 70% cancer deaths reported (WHO, 2018). In the present scenario anticancer agents used approximately 60% are natural sources. Out of these, approximately 25% are directly sourced from plants, while another 25% have been derived from plants through chemical modification or synthesis (Twilley *et al*. 2020).

Numerous methods are used for treating cancer such as surgery, radiotherapy, and chemotherapy as well as specialized techniques. In addition, herbal medicine used as complementary medicine for the treatment and management for cancer (Khan *et al*. 2019). Approximately 13% of fatal caused by liver, breasts, lungs, cervix, uteri, stomach and colorectal cancer annually across the globe (Omara *et al*. 2020).

**1.7. Anti-Inflammatory Activity**

Inflammation is an innate response, primary purpose of which is to remove harmful stimuli and begin the healing process. It is exemplified by the recruitment of immune cells to the site of injury, increased blood flow, and the release of various chemical mediators. The goal of inflammation is to eliminate the source of damage, remove damaged tissue and promote healing (Oguntibeju, 2018). Injury causes inflammation and it is a complex process that involves the start recruitment of various immune cells to the affected area. The primary goal of this response is to eliminate harmful agents and remove damaged tissue, ultimately leading to the healing of the affected area (Ginwala *et al*. 2019).

Heat, acid or base, organic solvents, or concentrated inorganic salts disrupts the protein structure, and this process leads to protein denaturation. This results in the loss of the protein's

normal function, as the active site for enzymes is no longer able to bind to its substrates. Nonsteroidal anti-inflammatory drugs (NSAIDs) used for medications to reduce pain and swelling. These drugs are also known to prevent protein denaturation, which leads to the formation of antigens and enhancement of autoimmune diseases (Dharmadeva *et al*. 2018). Due to the potential for drug-related toxicity and harmful adverse reactions associated with long-term use, synthetic anti-inflammatory drugs are becoming less desirable for treating inflammation. As a result, there is a growing need for the development of safer and more effective anti-inflammatory agents, drawing from herbal constituents and used for human illnesses (Hmidani *et al*. 2020).

**1.8. Angiogenic Activity**

The process of creating new blood vessels, known as angiogenesis, occurs through two distinct mechanisms: sprouting angiogenesis and intussusceptive angiogenesis (Vimalraj *et al*. 2019). Pioneered by Judah Folkman, the potential to clinically modulate angiogenesis has been demonstrated. Various assays have been developed to determine the effectiveness and efficiency of biomolecules in altering angiogenesis in human (Stryker *et al*. 2019). The CAM (Chorioallantoic membrane) assay is a widely used *in vivo* method for studying angiogenesis and anti-angiogenesis in response to various factors. It is simple, cost-effective and utilizes the dense capillary network of the chick embryo chorioallantoic membrane, making it an ideal model for *in vivo* studies. This assay played a crucial role in analysing anti-angiogenesis (Ribatti *et al*. 2020).

**1.9. Wound Healing Activity**

Wound is an inevitable aspect of human life. A wound is caused by physical, chemical, thermal, microbial or immunological factors and it affects the physiological function and anatomical structure (Thakur *et al*. 2011; Okur *et al.* 2018). Natural process for tissue repair involved coagulation, inflammation, cell proliferation, protein synthesis and remodelling. It aims to restore the skin's normal structure and functions after damage to the epithelial barrier and connective tissue. The ideal healing process may vary in duration but typically involves the formation of a platelet scab, inflammatory cell response, rapid proliferation of skin cells and structural repair (Muniandy *et al*. 2018).

Wound repairing is a complex process that involved four main stages: haemostasis, inflammation, proliferation, and tissue remodelling. These stages occur in a continuous cycle until the wound is fully healed (Alavi & Varma 2021). Acute wounds, such as those caused by

surgery or injury, happen suddenly and typically heal in a predictable period. However, if one or more stages of the normal healing process are disrupted, it can result in a chronic wound. They can be characterized by persistent inflammation, which can impede the normal healing process (Grada *et al.* 2018). Different drugs and ointments such as betadine, acetic acid, iodophor and hydrogen peroxide have been utilized in wound healing, each with its own set of limitations and drawbacks (Goorani *et al*., 2019). So, the discovery of natural remedies for enhancing wound healing is crucial both clinically and economically (Van de Velde *et al*. 2019).

Zebrafish is a freshwater aquarium fish, have become a widely used model organism in recent years for research in genetics, development, regeneration, and toxicology studies. Due to their special characteristics such as ease of care, high reproductive rate, swift development, small size, and rapid genetic manipulation. The sharing of resources and techniques within the zebrafish research community have played a significant role in the development of research utilizing Zebrafish (Meyers, 2018). The use of zebrafish larvae in drug testing has gained popularity as a cost-effective and efficient method for evaluating potential therapeutics. Zebrafish larvae offer a high-throughput alternative to more expensive mammalian models (Ellis *et al*. 2018).

**1.10. Molecular Biology**

Advancement in molecular research through the isolation of high-quality DNA, sequencing, cloning, to produce a new variety of species and genetically modified organisms. The standard protocol for extracting DNA from various plant seeds and crops, belonging to seven different orders, is based on the modified version of the commonly used cetyl trimethylammonium bromide (CTAB) method (Aboul-Maaty & Oraby, 2019). Obtaining high-quality DNA from plants is a challenge due to the presence of several contaminants that are present in the rigid cell wall and can interfere during the DNA extraction process (Heikrujam *et al*. 2020). Commercial DNA isolation kits have become the most popular choice for DNA extraction in current times (Shipley *et al*. 2022).

Polymerase chain reaction (PCR) is utilized in cases where enhanced sensitivity and specificity are required, such as when amplifying a specific member of a polymorphic gene family or when amplifying a low-abundance mRNA, cDNA in a clinical sample containing a diverse cell type population (Green & Sambrook, 2019). PCR to selectively amplify a nucleotide sequence from a DNA extract, a minimum of one pair of oligonucleotides is required. These

oligonucleotides serve as primers for replication and are chemically synthesized to ensure optimal complementarity with both ends of the desired sequence. One of the primers recognizes the complementary sequence of the 5' to 3' fragment, while the other recognizes the same fragment in the 3' to 5' direction. These primers are single-stranded DNAs, which hybridize to the sequence flanking the target sequence, allowing for its selective replication. To ensure specific hybridization on the DNA matrix, the primer size is typically between 10 and 30 nucleotides (Kadri, 2019).

Transformation is an essential process in biotechnology that enables the transfer of exogenous DNA materials into bacteria. It is an efficient, user friendly, rapid, cost-effective, and flexible enough to be applied to a diverse array of microbial species. Although artificial transformation techniques have been widely used in laboratories and are readily applicable to *Bacillus subtilis* and *Escherichia coli*, conventional methods may have limited efficiency for newly exposed bacterial species (Ren *et al*. 2019).

A genome is the complete genetic information of an organism and stored in nucleic acids. Nowadays advanced technologies have developed to identify the gene sequence. The annotation of genomes allows for the comprehension of gene function. Furthermore, DNA sequencing is used to analyse the molecular evolution (Giani *et al*. 2020).

### 1.11. Molecular Docking Studies

Molecular docking is a popular structure-based approach in drug design that predicts the interaction between receptors and ligands by simulating their molecular interaction. This method has gained widespread use in the drug design research field due to its ease of use, cost-effectiveness and efficiency. Screening potential pharmacophores through compound databases enables researchers to efficiently purchase, synthesize and conduct follow-up pharmacological tests. The development of reverse molecular docking technology has also significantly improved the ability to predict drug targets and understand the molecular mechanisms involved in drug design (Fan *et al*. 2019).

The process of molecular docking involves two key steps: sampling and scoring. The sampling step involves searching for possible conformations of the molecules being docked, considering the flexibility of both the receptor and ligand. To manage this large conformational space, the receptor is often kept rigid. The scoring function assesses the fitness of each sampled pose. It determines which conformations will be kept from the sampling process and orders them

based on their likelihood of being accurate. The result of molecular docking is a list of docked molecules arranged in order of their potential accuracy, based on the ranking given by the scoring function. This ranking helps to identify the most likely binding mode between the receptor and ligand molecules (McNutt *et al*. 2021).

An integrative *in silico* approach has been developed in the field of network pharmacology, which aims to target disease mechanisms as networks by using multiple synergistic drugs (Casas *et al*. 2019). The information age progresses, a range of network technologies are being developed. Network pharmacology, which merges pharmacology and information networks and is based on system biology, bioinformatics, and high-throughput histology, is becoming increasingly popular (Zhou *et al*. 2020).

## AIM AND OBJECTIVES

To overcome the lacuna in enhancing seed propagation, an efficient strategy is strongly required. Hence, we have been proposed a method for propagation as well as conservation and understanding the molecular basis of alkaloid biosynthesis. Based on the ethnopharmacological study *Rauvolfia tetraphylla* is used to treat wound in folk medicine. Despite the recognized potential, there remains a dearth of studies exploring the wound healing capabilities of this plant extract. Consequently, this research has the potential to address the therapeutic gap crucial for effective wound healing.

- To optimize the seed germination potential for *Rauvolfia tetraphylla*, and to investigate the effect of PGR on callus induction, root and shoot formation from different explants, subsequently examining the histological aspects of somatic embryogenesis derived from the callus.
- To identify and characterize the major bioactive compounds in *Rauvolfia tetraphylla* using spectroscopic chromatographic techniques.
- To assess the biological activity of various crude extracts from leaves and fruits from *Rauvolfia tetraphylla* for assessing wound healing properties.
- To isolate and characterize the Tryptophan Decarboxylase (TDC) gene from *Rauvolfia tetraphylla* (*RtTDC*), investigating its sequence, structure, catalytic regions, evolutionary relationships, physicochemical properties, and substrate recognition, followed by experimental validation of its catalytic activity through a TDC assay.
- To perform docking analysis of the bioactive compounds of *Rauvolfia tetraphylla* against a wound healing protein (6Y8M, 6B8Y and 1GEN).