

CHAPTER - I

INTRODUCTION

1.1. General Introduction about Medicinal Plants

Plants served as a significant resource for food, medicine, fertilizer, shelter, and clothing (Mulugeta et al., 2024). Nowadays, people's attention to medicinal plants were increasing due to universal pandemic, they have concerned about their health and persistently sick people are desiring herbal medicines to boost immune system (Panjiyeva & Elmurodova, 2024). However, 85% of the world population sees medicinal plants to be primary healthcare and source for drug discovery (Fitzgerald et al., 2020).

The uses of medicinal plants have various beneficial effects which is commonly used to treat illness, it has been practiced over the generations. Salmeron et al., explained about folk medicine was knowledge and practices based on the beliefs of indigenous people which is used to prevent physical and mental illness (Salmerón-Manzano et al., 2020). In global healthcare utilization of natural products and their derivatives, contributing to over half of the medications throughout the world (Shedoeva et al., 2019). Due to climate change, over-harvesting and habitat loss can affect the conservation of these plants. Insufficient conservation of medicinal plants can impact their yield (Roopashree et al., 2024).

The evolution of modern medicine can offer lifesaving solution, yet it has a great challenge to treat side effects. Therefore, herbal medicine can be used as an alternative remedy (Abubakar & Haque, 2020). For decades, medicinal plants produce natural products which have diverse group of bioactive compounds are alkaloids, phenols, saponin, terpenoids, tannins etc., to treat various disease (Cedillo-Cortezano et al., 2024).

The gaining of ethnomedicinal knowledge in biosciences is to explore novel bioactive compounds and create plant extract formulations for various pharmaceutical purposes. Drug discovery is a tedious process and should select suitable method depends upon the selective bioactive compounds. Reverse ethnopharmacology was introduced by Süntar, in which the unveiled bioactive compounds were discovered from the folk medicinal plants and their biomedical applications were demonstrated (Süntar, 2020).

1.2. General Description About Apocyanaceae

The family Apocynaceae encompasses approximately 378 genera and 5,350 species, predominantly distributed across pantropical regions, with select genera extending into temperate zones *Cynanchum tunicatum* (Retz.) Alston is one among them (Endress et al., 2018). In Chinese medicine, *Cynanchum* species is used to treat fever, cough, pneumonia, and asthma due to the presence of various phytoconstituents. Literature survey of *Cynanchum* species indicates the existence of approximately 450 compounds, which includes steroids, alkaloids, terpenes, flavonoids, polysaccharides, and steroidal glycosides (Han et al., 2018). In the meta-population theory, the degradation of natural wildlife habitats holds the potential to drive the extinction of numerous species globally due to the loss of sub-populations (Hanski et al., 1995). Considering the phytochemicals present in the *Cynanchum* species, there will be explored prodigious bioactive compounds existing in the *Cynanchum tunicatum*.

1.3. *In vitro* Propagation

Plant tissue culture is efficient for producing genetically identical plant species (Syed et al., 2021), owing to increasing the demand of phytoconstituents in medicinal plants. It influences in restoration of therapeutic plants through cell and organ culture (Dogan, 2019). Organogenesis and somatic embryogenesis are key pathways for micropropagation, and it leads to the establishment of new plants through somatic cells (Liu et al., 2023). The micropropagation of medicinal plants is a highly effective technique for plant breeding, crop improvement and it offers several advantages compared to conventional method (Basha et al., 2022). It could be a great choice for multiplying, regenerating and accumulating bioactive compounds at restricted conditions (Usman et al., 2020). The *in vitro* callus could achieve the production of desired natural products with the help of elicitors (Asyakina et al., 2021). Transgenic plants have been established an effective regeneration protocol (Yu et al., 2021). Plant tissue culture is a subset of biotechnological tools which effectively contributed into two key areas such as commercial implement of sterile plants and preservation of rare and endangered plant species (Sharma et al., 2021).

1.4. Seed Culture

Seed culture is the essential for conserving RET listed medicinal plants. Commonly, plants reproduced by seeds, some plants have recalcitrant, dormant, low viability seeds, and to overcome these problems through *in vitro* seed culture. The *in vitro* seed culture method is

simple and economically feasible for commercial applications. This method is efficient for overcoming seed dormancy and low germination percentage.

1.5. *In vitro* Callus Culture

The callus is a homogeneous cells produce a mass of which has a potential to continues cell division. The callus has raised from wounded sites of different explants. Callus induction based on the various factors such as explant type, growth hormones and incubation period (Salih et al., 2020). Several recalcitrant plants such as lotus, tobacco, soybean, *Arabidopsis*, rice and tomato have different regeneration protocols to conserve such plants (Deng et al., 2020). The appropriate concentration of growth regulators is essential, as different plant species have unique requirements for optimal callus growth. The proper ratio of auxin and cytokinin into callus medium facilitates the callus initiation (Zaman et al., 2020).

1.6. Somatic Embryogenesis

Somatic Embryogenesis originate from corporal cells aligned with zygotic embryos distinct stages and it has potential to produce a young plantlet resembling mother plants. These embryos can easily proliferate into embryonic cell suspension and then regenerate into numerous young plantlets (Adero et al., 2023). The stages of somatic embryos were recorded as globular, heart-shaped, torpedo, and cotyledonary embryos. This technique offers an alternative way to conventional breeding for clonal propagation (Hao et al., 2023), with a high reproduction rate, strong genetic stability, and uniformity in regenerated individuals (Bai et al., 2023).

1.7. Phytochemistry

The medicinal plants have various biomolecules are of phenolics, flavonoids, saponins, terpenoids, and alkaloids (Alruwad et al., 2023). Especially, the bioactive compounds were responsible for the *in vitro* biological activities (Mudau et al., 2022). Bioactive compounds were analysed by extraction, phytochemical screening, isolation, characterization, toxicological and clinical evaluation (Sasidharan et al., 2011).

1.8. Antioxidants

Free radicals are considered as the key factors in many chronic and cardiovascular diseases (Zhang et al., 2023). Natural antioxidants were present in plants in the form of phenolic compounds which prevents the oxidative damages (Wan et al., 2022). The antioxidant capacity

of polyphenols can also be used as an anti-aging agent, free radical scavengers, and inactivators prooxidants (Moliner et al., 2020; Abeyrathne et al., 2021; Zhao et al., 2021). Phenolic compounds and carotenoids are rich source of antioxidants (Stobiecka et al., 2022). Antioxidants are significant in neutralizing harmful free radicals and reactive oxygen species (ROS) and maintaining homeostasis of human body (Rahman et al., 2021).

1.9. Anti-Microbial Activity

Antimicrobial activity plays a crucial role in intensive farming by treating and preventing infections. Additionally, microbes are utilized in animal feed to promote growth (Lhermie et al., 2018). The infectious diseases are challenges across the global, to overcome the issue discovered by many antimicrobial drugs from the microbes and plant extracts (Manandhar et al., 2019). Natural antimicrobial agents, whether employed on their own or in combination with antibiotics, can augment activity against a diverse array of microbes. As the antimicrobial potential of many medicinal plants still awaits comprehensive exploration, scientists are actively engaged in novel and potent treatments (Vaou et al., 2021).

Nevertheless, historical patterns indicate that newly introduced antimicrobial agents quickly encounter widespread resistance. This emphasizes the necessity for innovative solutions with greater longevity (Manandhar et al., 2019). Previously identified numerous antimicrobial compounds from synthetic and natural sources to treat infectious agents. The rise of multidrug-resistant bacteria has worsened issues of accessibility and affordability for commonly prescribed antibiotics, leading to reduced treatment effectiveness, increased levels of morbidity, mortality, and healthcare costs (Kebede et al., 2021). Therefore, the use of natural antioxidant and antimicrobial compounds derived from plants are practiced (Sharma et al., 2020). Internationally, essential oils and extracts derived from plants are investigated as potential property of phytochemicals and inovated of antimicrobial compounds, food preservatives, and alternative for various infectious diseases (Hadadi et al., 2020). The discovery of antibiotics is a pivotal achievement in modern medicine, saving millions of lives (Karas et al., 2020).

Plant-derived antimicrobial compounds are being safe due to their natural origin, and promising source compared to synthetic compounds. Plants possess bioactive compounds widely applied in clinical practice, and a notable number of drugs in the market originate from natural resources (Mickymaray, 2019). Plant-based derived products are served as cost-effective alternative to the development of synthetic drugs (Raja Ratna Reddy et al., 2020).

Uncovering new sources of antimicrobials is crucial in the face of growing antimicrobial resistance (Mohammed et al., 2020). Antimicrobial resistance possesses worldwide threat to all kind of bacterial pathogens (Bhatia et al., 2021). Plants have been vital to human health, producing biologically active substances in response to pathogens and environmental stress (Nunes et al., 2020).

1.10. Anti-Inflammatory Activity

The epidemiological studies suggested the targeting inflammation and its molecular components might be serve as a valuable approach for both preventing and treating cancer. Inflammation impacts tumour development and progression (Zappavigna et al., 2020). Its defence response of body cells against sudden injury, infection and persistent in acute pain, accompanied by fever, unexpected weight loss, fatigue. Inflammation contributed to the factors from both genetics and the environment, including diet (Maleki et al., 2019). It paves crucial role in signalling and protecting against foreign invaders (Yadav & Mohite, 2020). Since centuries, herbal therapeutics and dietary supplements are increased for alleviating inflammation and pain (Yesmin et al., 2020). Inflammation involves intricate vascular tissue reactions to harmful stimuli, often linked to pain, resulting in protein denaturation, membrane modifications, and increased vascular permeability (Pradhan et al., 2021).

1.11. Anti-Cancer Properties

Cancer is one of the foremost reasons for global death. About 70% of rapid deaths arise because of cancer and it is rising rapidly in Asia, Africa, Central and South America. Numerous research focused on anticancer therapies Cancer is described as an uncontrollable development and spread of anomalous cells forms a cluster. Until the cell formation is not controlled, it is considered as abnormal cell proliferation which leads to death. Mostly it is treated with surgery, chemotherapy or radiotherapy (Nguyen et al., 2020; Malaikolundhan et al., 2020; Septisetyani et al., 2021). Plant-based compounds show promise, and most of the medicinal plants possess bioactive compounds with anticancer properties, and their extracts have been used in both traditional and modern cancer treatment.

The case study reported the new cancer cases raises to 29 million by 2040 based on Global Burden of Cancer study because of increasing population and other socio-economic factors. Mostly 55% of the new cases reported in developing nations, the herbal and traditional medicines are used to gain energy, boost immune system and also prevent various diseases (Oladeji et al., 2024). Huang described the folk medicine used to delaying the growth of cancer

even though lack of research findings. Therefore, most of the plant species require research support for their application in folk medicine. Phytochemicals contribution to the treatment of cancer by discovering new therapeutic compounds (Huang et al., 2024).

Cynanchum tunicatum is a rare medicinal plant, it needs to be conserved using plant tissue culture method. Optimization is required for *C. tunicatum* yet this species is novel for *in vitro* propagation method. So, standardization of abiotic factors is crucial for the development of callus and organogenesis.

1.12. Aim and Objectives

Cynanchum tunicatum is a rare medicinal plant belongs to Apocynaceae family. To conserve this plant species from threaten, *in vitro* micropropagation was used through plant tissue culture. This study explores the novelty of this species, including standardization for *in vitro* callus induction, plant regeneration, phytochemical compounds, and various *in vitro* biological activities.

- ✓ To develop and optimize the culture conditions for *Cynanchum tunicatum in vitro*.
- ✓ To determine the phytochemical profiling of *Cynanchum tunicatum*
- ✓ Comparative study of biological activities of wild and *in vitro* callus of *Cynanchum tunicatum*.
- ✓ To analyze the *in-silico* studies of bioactive compounds of *Cynanchum tunicatum*