CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1. Background

Azadirachta indica is known as the neem tree and is a member of the Mahogany family Meliaceae. It is one of two Azadirachta-type species and comes from the Indian Subcontinent and most of African countries. Neem extract metabolites are limonoids, tannins, alkaloids, terpenoids, catechins, sterols, gallic acids, and sugar reduction. Neem is a rich source of limonoids, antioxidants, anti-inflammatory, and anticancer activities, with powerful health benefits. **(Akinloye *et al.*, 2021).** Limonoids are high oxygenation triterpenes that have lost four carbon atoms of the lateral chain and are consequently tetranortriterpenoids, with the remaining lateral chain being cyclical into a furan ring. It is a natural pentacyclic secondary metabolite**(Braga *et al.*, 2020).** The chemical synthesis and biopsal characteristics of this molecule have continually been investigated since the first isolation and characterization of this substance in the 1960s **(Ong *et al.*, 2021).** The herb has traditionally been a popular medicine for treating malaria and other illnesses. Evidence studies indicate a tremendous potential for the treatment of cancer, neurological and infectious illnesses of gedunin and their products. Several biological actions, including antibacterial, insecticidal, anti-malarial, anti-allergic, anti-inflammatory, anticancer, and neuroprotective, have been linked to gedunin **(Mukherjee *et al.*, 2008).**

Compared with other neem limonoids, azadirachtin, gedunin & nimbolide are more thoroughly studied. Cumulative data show that the benefits of neem limonoids against cancer can be mediated through the suppression of distinctive

malignant characteristics, such as apoptosis, cell proliferation, inflammation, invasion, and angiogenesis. Neem limonoids mainly target the NF-μB, Wnt/β catenin, PI3K/Akt, MAPC, and JAK/SAT signaling pathways for oncogenic signaling kinases and transcription factors **(Patel *et al.*, 2016).**

Gedunin $C_{28}H_{34}O_7$ functions as a control method in prostate cancer for androgen receptor activation, almost 80% reduction in ovarian cancer cell growth following in-vitro gedunin therapy. In human ovarian cancer cells during the G2/M phase, gedunin induced severe ROS damage and cell cycle arrest, thereby limiting cell growth. Overregulation of ROS also led to a depolarization of the mitochondria and membranes that ultimately caused mitochondria-mediated apoptosis after the release of Caspase9, 3, and cleavage of PARP **(Sahai *et al.*, 2020).**

Traditional plant-based treatments could offer a potential path to venom neutralization. India has a rich biodiversity with many plant species, which might be ideal candidates for successful snakebite treatment by medicinally-important plants. Literature research has demonstrated the success of various active natural compounds produced from *Aristolochia indica* plant species. *Withania somnifera* and *Azadirachta indica* extracts reduce inflammation, tissue damage, and severe pain in local regions. These plant species are active substances that act upon and prevent the action of specific enzymes present in venom. Serpentine phospholipase A2 is one such enzyme **(Gomez-bentacur *et al.*, 2019).**

The term "antidiabetic agents" refers to a wide range of medications, synthetic as well as natural, used to treat type II diabetes. There are now six kinds of oral diabetes medicines (OADs) available: biguanides, sulfonylureas, thiazolidinediones, dipeptidyl peptidase IV inhibitors, and alpha-glucosidase

inhibitors (e.g., acarbose) **(Sultan *et al.*, 2022).** Current medications include adverse effects such as hypoglycemia, and weight gain, necessitating the development of novel antidiabetic targets and therapies for glycemic control. The inadequacy of current medicines to manage hyperglycemia without causing adverse effects, as well as their high cost and scarcity, drives the search for traditional herbal remedies that may give useful leads and therapeutic solutions. HPA inhibitors have also been reported to be free of negative effects **(Haldar *et al.*, 2015)**. The use of natural plant products as an alternative approach to diabetes therapy is increasing. Gedunin shows ant-diabetic activity against alpha-amylase and alpha-glucosidase in vitro by inhibiting via non-competitive and mixed inhibition of the enzyme.

1.2. Motivation and significance of research work

Tetraterpenoids derived from natural resources have high nutritional value and great structural diversity and are economically feasible and motivated to search for new lead compounds for drug discovery. Neem is the most utilized plant in India and its subcontinents.

In the computational interaction of gedunin and 5" nucleotides, metalloproteinase-like snake venom enzymes were validated through computational biology approaches in the present studies on molecular dynamic simulation, whereas in silico and in vitro studies were carried out to study the anti-diabetic and anticancer potential of gedunin.

Gedunin modification of the enzyme pocket revealed that modified gedunin is a better candidate than snake venom enzymes. These findings are valuable for understanding the behaviour of gedunin and modified gedunin's presence of 5 "nucleotidase, metalloproteinase-like enzymes.

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Gedunin-based research may be helpful in the development of anticancer and anti-venom with high potency, efficacy, safety, and purity. Therefore, an attempt was made to achieve the following objectives.

1.3.Objectives

1. Extraction, purification, and characterization of gedunin from *Azadirachta indica*.

2. *In silico* evaluation of gedunin from *Azadirachta indica* as anti-venom agents.

3. Modification of gedunin and *in silico* study of modified inhibitor.

4. QSAR and Retrosynthesis study of $C_{26}H_{31}N_2O_6F$ (modified gedunin) inhibitor.

5. Molecular property prediction of $C_{26}H_{31}N_2O_6F$ (modified gedunin) using Machine Learning.

6. *In vitro* and *in silico* evaluation of gedunin from *Azadirachta indica* as an anti-diabetic agent.

7. *In vitro* and *in silico* evaluation of gedunin from *Azadirachta indica* as an anticancer agent.

8. Transcriptomics analysis of gedunin.