CHAPTER 11

Summary and Conclusions

The study sought to ascertain the therapeutic benefits of gedunin derived from Azadirachta indica. India is abundant in both vegetation and animals. Neem is used as a medicinal herb in the Indian subcontinent since ages. The current study investigates the anti-venom, anti-diabetic, and anti-cancer potential of a bioactive constituent (gedunin) extracted from neem.

The most powerful antidote to snake venom is ASV, which is composed of purified IgG fragments from horse or sheep plasma or serum vaccinated with snake venom. It can be monovalent, like CROFAB, which helps against rattlesnake, copperhead, and cottonmouth poison. ASV is not used on a regular basis due to its scarcity, and the unique activity of the species, as well as storage issues, contribute to its minimal utilisation. The fundamental issue with immunotherapy is its specificity, which varies greatly between species and geographical locations, limiting the use of particular ASVs. Furthermore, owing to a lack of knowledge about the geographical variety of toxic plants, it cannot be raised against all species and subspecies; therefore, gedunin and modified gedunin $C_{26}H_{31}N_2O_6F$ can be used as an alternative antidote against snake bite as they show activity against major enzymes phospholipase A2, metalloproteinase, 5‟nucleotidase, L-aao, acetyl cholineastrace with the binding energy of -10.60, -9.00, -15.90, -13.60, -10.60 kcal/mol for gedunin and -9.30, -8.40, -14.80, -10.60, -9.90 kcal/mol for modified gedunin $C_{26}H_{31}N_2O_6F$. Diabetes mellitus (DM) is elevated blood sugar levels, and oxidative stress is a significant etiology in DM, resulting in oxidative stress. Gedunin contains anti-

oxidative properties and might be an alternative treatment for diabetic patients. *In vitro* studies have shown that gedunin binds to alpha-amylase and alpha-glucosidase enzymes at allosteric sites to form hydrogen bonds, salt bridges, and hydrophilic interactions. Many synthetic drugs are available to treat diabetes; however, these have numerous side effects hence gedunin can be the alternative medicine to treat type II diabetes.

The nature and type of solvents used in the extraction and purification of components from A. indica vary; gedunin was extracted using soxhlet and non-soxhlet techniques, while the sample was purified using column chromatography and characterized using HPLC and HRLC-MSQ-TOF. The presence of gedunin in the seed extract was identified using HPLC screening. The presence of gedunin in seed extract was verified by HRLC-MSQ-TOF.

Knowledge of the interaction between gedunin and anti-diabetic, anti-cancer, and anti-venom enzymes is required to create effective medicine. The molecular docking technique may be used to analyze the interaction between a small molecule and a receptor at the atomic level, which can provide insight into characterizing the behaviour of minor compounds in target protein binding sites and elucidating essential biochemical processes. The docking investigation revealed that the van der Waals, electrostatic, and desolvation energies all play a role in binding.

QSAR models were created using machine learning algorithms such as random forest and DNN (deep neural network), as well as 5-fold cross-validation, to predict log P and log $_{IC50}$ using descriptors from CDK2.0 in the case of DNN and molecular weight, log P in the case of random forest, and a molecular data set similar to the inhibitor molecule. Log P IS 2.97 kow, and Log $_{IC50}$ is 7.17 (-log M unit) and 0.70 (-log M unit) of gedunin, respectively. With an R_2 near to 1 and a

low RMSE value, the log-P and log-IC_{50} models fit well. The retrosynthesis of modified gedunin revealed a unique synthesis process that did not include gedunin as a reagent or intermediary.

According to the previous investigations, it is clear to anticipate the chemical features of freshly created compounds using smiles as input data and the K-MEANS algorithm using deep learning (CNN and transfer learning). Almost 5000 molecules were used for training, and vggnet was used for feature extraction. Clusters were created using pre-exercise data and the newly produced grin of modified gedunin based on the similarity of attributes based on LST/RNN ($C_{26}H_{31}N_2O_6F$).

Lipinski's five rules were used to identify Gedunin as a medication candidate. Gedunin's molecular docking and simulation research to function as an inhibitor of the 5'Nucleotidases snake venom enzyme showed strong pharmacokinetic, physicochemical, and drug-like qualities that may be obtained using the Stardrop software ICM. molsoft and Swiss-ADME. The chemical is a P3A4 isoform substrate with potential skin, ocular irritation, sensitization, developmental toxicity, and hepatotoxicity effects. The molecule's modification reduced the BBB log, log P, and log D probability values while raising HBA and HBD. When a molecule is delivered intravenously or orally, it is less likely to reach the CNS.

The modified gedunin $C_{26}H_{31}N_2O_6F$ as antivenom was used as the lead candidate for further drug development process as a treatment for snake venom. Simulation studies revealed that gedunin binds to snake venom key enzyme 5'Nucleotidases at its binding site, forming an energetically stable compound.

Gedunin's IC_{50} result in the MTT test of the HepG2 cell line was 35.95 g/ml. Gedunin and H_2O_2 therapy increased ROS levels in liver cancer cells by decreasing

the level of ROS from 53.7 to 36.4. Reactive oxygen species are involved in a wide range of biological functions. Depending on the degree and length of exposure, cell survival and death pathways can result in high ROS levels, which can induce cell damage and oxidative stress. In multicellular organisms, apoptosis is a sort of planned cell death (from ancient Greek to ancient reco to the apoptosis of spring). Apoptosis is another name for programmed cell death. Biochemical processes cause distinctive cellular changes (morphology) and cell death. Gedunin also boosted the expression of active Caspase-3 in HepG2 liver cancer cells, PA1 and PC3 cells *in vitro* with caspase activity of 41.1, 57.4 and 47.9 fold increase, respectively and *in silico* using caspase 3 receptor as well as CEA receptor present in both PA1 and PC3 cell lines showing lowest binding energy of -9.15 and -7.96 kcal/mol at active site Scratch assay or migration assay of NIN/3T3 (non-cancer cell line) cell line showed complete cell migration in the presence of gedunin from 0 hour to 24 hours whereas in HepG2 cell line 46.21 % cell migration was seen in control and 2.46 % cell migration after 24 hours of gedunin treatment (36 µg/ml gedunin). In PA1, 100 % cell migration was seen in control and 15.26 % cell migration in 74 µg/ml gedunin-treated cells after 24 hours. Furthermore, in PC3 92.63 % cell migration was seen in control and -0.54% in 96 µg/ml gedunin-treated cells after 24 hours. In colony formation assay/cell proliferation assay the control showed 1 surviving fraction in the case of HepG2, PA1 and PC3 cell lines whereas 0.96, 0.36 and 0.33 surviving fractions after 24 hours in the case of HepG2, PA1 and PC3, respectively, hence gedunin can suppress proliferation of anti-cancer cells. In vivo, investigations demonstrate that gedunin-treated Hela cells pass the QC test and express 35353 cells, with 92 genes downregulated and 24 genes upregulated. Gedunin is implicated in 13 KEGG pathways, including the PI3K-Akt signalling pathway,

central carbon metabolism in cancer, the formation of neomycin, kanamycin, and gentamicin, the interaction of ECM with receptors, and others. Thus gedunin has been proved to be a potential anti-cancer agent against human cervical cancer cell line.