CHAPTER 4

COMPUTATIONAL ANALYSIS OF A GEDUNIN AS A POTENTIAL INHIBITOR OF SNAKE VENOM ENZYMES

4.1. Introduction

Snakebites have become a public health concern in India. In a single year, more than 250,000 snakebites are documented. In terms of length and body weight, snakes in India have a wide range of species. Snakes live in deserts, woodlands, marshy areas, lakes, streams, and rivers in difficult terrain **(Longbottom *et al.*, 2018).** Bites not only lead to death but are also responsible for severe paralysis, irreversible kidney failure, permanent amputation of limbs, and disability. Antidotes can only be administered if the species is known or believed to be responsible for the bite, and the first six hours are critical. Therefore, an antidote should be administered during this period to protect the victim from toxic effects and death.

The plant kingdom is a source of various plant compounds that are effective against snake venom **(Gupta *et al.*,2012).** Traditional healers and compounds use various plants extracted from plant sap, roots, leaves, etc., in the form of powders, pills, pastes, etc. This can be useful in remote areas of India and other parts of the world. Plants are regaining a new form owing to their effectiveness, abundance, and safety. **C. Venkatesan *et al***. reported that plants could be used as an alternative to antiserum and against poison enzymes such as 5'-Nucleotidase, phospholipases, hydrolases, metalloproteinase, amino acid oxidase, and Acetylcholinesterase, which are commonly found in all species of snakes.

Neem (*Azadirachta indica*), Indian lilac, or margosa, has been known in India and neighbouring countries for more than 2000 years and has a history of being used in medicine in one way or another. Taxonomically, the neem (*Azadirachta indica*) belongs to the Meliaceae and Meloidae subfamilies.

The bioactive compounds that make neem very popular are salannin, Nimbin, meliantriaol, tetraterpenoid, flavonoids, gedunin, saponin, tannins, quercetin, gallic acid, limonoids, azadirachtin and all of these belong to the limonoids, sterols, stigmasterol classes, **(Hoda *et al.,* 2015).** These bioactive compounds are found in different parts of neem like seeds, leaves, fruits, and root extracts, and the possible effects when we use these extracts can be molecular mechanisms, cellular mechanisms, programmed cell death, autophagy, DNA repair, inhibitory effects, anti-inflammatory, detoxifying, anti-metastatic activities, immune surveillance, anti-angiogenic and so on **(Islas *et al.,* 2020).**

Heavily oxidized triterpenoids were isolated in 1968. The secondary metabolite of neem took 17 years to predict its structure **(Schaff *et al*., 2000).** Tetraterpenoids such as gedunin can be extracted from neem fruit or oil and have been shown to act as anticancer agents, antimalarials, and insecticides because of the variety of chemical surfaces inhibiting Hsp90 proteins. Each offers regioselectivity **(Brandt *et al*., 2008).** Gedunin is a multi-target compound that also modulates pathogen-associated molecular patterns (PAMPs) and triggers an anti-inflammatory reaction via heme oxygenase and IL-10 production, which increases the therapeutic efficiency of gedunin **(Costa *et al.,* 2020)**.

Here in computational analysis of a gedunin as a potential inhibitor of snake venom enzymes, Molecular docking and ADMET studies were performed using the ICM

Molsoft and Stardrop software, which are more reliable and accurate than autodock. These methods are based on the Monte Carlo method.

4.2. Experimental

4.2.1. Computational methods

4.2.1.1. Molecular Docking

Molecular Docking is possible in two connected steps: the first is sampling the ligand in the protein's active site; this is then ranked using a score function. Sampling methods should ideally replicate the experimental binding mode and be ranked among all generation conformations with the highest sampling function**(Morris *et al.*, 2008)**. Flexible ligand docking was performed using ICM software in the potential map grid derived for the enzyme active site pocket. The inhibitor gedunin was docked to snake venom enzymes, namely, 5'Nucleotidases (5'NT), Acetylcholine, L-aao (L-amino acid oxidase), metalloproteinase, phospholipase A2, and thrombin-like hydrolase. Binding modes for poses that are ordered according to the score function prioritize the top poses.

4.2.1.1.1. Lig and Preparation

The ligand gedunin (PubChem ID: 12004512) was made by converting it into an ICM object by the depletion of water and optimization of hydrogen, his-pro-asn-gln-cys, carried out outside the pocket of the enzyme (receptor) **(Arnautova *et al.*, 2018).**

4.2.1.1.2. Receptor Preparation

The docking receptor file was downloaded from the RSCB PDB with PDB ID:5H7W,3KVE,1ND1, 7M6C, 1FSS, and receptors (poison enzymes) were produced by optimizing hydrogen and missing side chains, removing excess water

bound to receptors, and converting the PDB coordinates to ICM internal coordinates **(Ballante *et al.*, 2021).**

4.2.2 Simulation

Gromacs used for MD (Molecular Dynamics) used the concept of a periodic boundary to create boxes/grids and groups to show the action in the OPLS force field for 50 ns **(Rawat *et al*., 2021).**

4.2.3. ADMET

ADMET (Absorption, Distribution, Metabolism, Excretion, and toxicity) drug properties are at the heart of the drug development process because access to physical samples is minimal. These were predicted using SwissADME**(Alqahtani*et al.*, 2017).**

4.2.4. Toxicity

Toxicity prediction (LD50) for oral administration using the web-based server, Protox-II **(Jackson *et al.*, 1995).**

4.3. Results and Discussion

4.3.1. Molecular Docking

The docking **(Longbottom *et al.*, 2018)** was carried out with the ICM molsoft software, and the results for the investigated gedunin are shown in **Table 4.1.,** where the L-aao enzyme ligand complex has at least -15.90 kcal/mol binding energy, suggesting that the inhibitor spontaneously binds to the L-aao enzyme active site. The binding energy residues of the ligand enzyme complex are -10.60, -9.00, -13.60, and -10.60 kcal/mol for *5″* Nucleotidase, Acetylcholinesterase, metalloproteinase, and phospholipase A2, respectively. The binding energy is released when the

receptor associates with the ligand. A negative value indicates that the interaction is spontaneous; thus, a higher (-) value corresponds to better interaction. **Figure 4.1.** shows the binding site of the 5'-nucleotidase enzyme and its binding to gedunin, causing interactions with the site and collisions. Hydrophobicity or lipophilicity is thought to be a measure of a Ligand preference for a nonaqueous environment over an aqueous one. The buriedness was calculated using receptor density. It is a metric of evolutionary change. The aromaticity of the receptor is described as a feature of conjugated cycloalkenes that increases a molecule's stability owing to electron delocalization in the - orbitals. Aromatic molecules are thought to be particularly stable as they do not easily break and react with other types of chemicals **(Taylor *et al.*, 2008).**

The selection of enzyme docking sites was based on the DLID (Drug like density) likelihood of each pocket in the enzymes, and buried pockets influenced this drug rating. The DLID (drug-like density) metric assesses the likelihood of a pocket binding a drug-like molecule. That was estimated by adding the number of additional pockets in its immediate vicinity that contain drug-like co-crystallized ligands to the total number of pockets in the area **(Holloway *et al.*, 2010).** In the active area of the enzyme, the DLID probability was the highest, with a reference DLID score of 0 -1. **Table 4.2.** shows the DLID pocket burial of the enzymes.

Table 4.1 Gedunin docked with snake venom enzymes. Lower binding energy indicates good docking of the drug against the target.

SNAKE VENOM ENZYME	BINDING ENERGY (Kcal/mol)
5'NUCLEOTIDASE	-10.60
ACETYLCHOLINESTERASE	-9.00
L-AAO	-15.90
METALLOPROTEINASE	-13.60
PHOSPHOLIPASE A2	-10.60

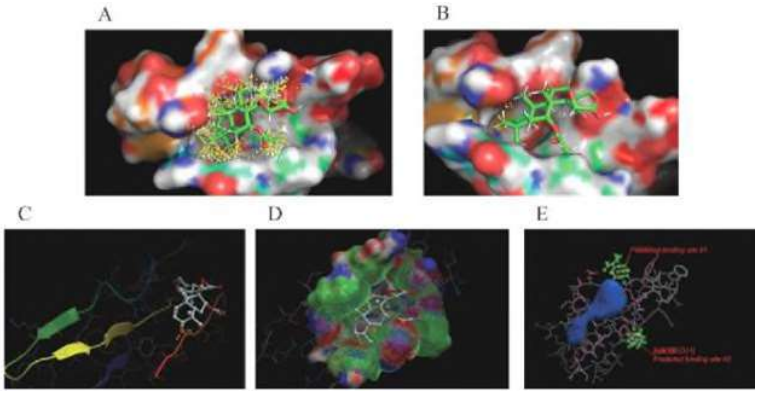

Figure 4.1 Gedunin and snake venom enzyme 5'NT interaction in the enzyme pocket depicting drug clashes at the target site (A, B, C, and D). The target site has the highest DLID score: hence it is an active site (E).

Table 4.2. Illustration of Receptor pockets/ligand interaction site's pocket volume, area, Aromaticity, hydrophobicity, and druggness probability. Buriedness of pocket ranges from 0 to 1 (open to wholly buried).

Enzyme	Pocket	Volume $(m)^3$	Area $(m)^2$	Hydrop hobicity	Buriedness	Aromaticity	DLID prob*	Non-sphericity
Acetylchol inesterase	1	239.2	299.3	0.5	0.7	0.1	0.03	1.6
Metallopro teinase	1	239.5	252.9	0.5	0.7	0.1	1	1.3
5' Nucle otidase	1	164.9	198.2	0.4	0.6	0	0.71	1.3

4.3.2. Simulation

Molecular dynamics is a method used to study the structure of compounds/solids using classical mechanics and to create a trajectory. Gromacs was used for MD, which uses the concept of a periodic boundary to create boxes/grids and groups to show the action. Simulation studies of 50 ns show that the average angle between Gedunin and 5'NT enzyme was between 70-760, and density was between 980-984 kg / m3. The radius of gyration decreased from 1.05 to 0.95-1 after complex formation. The number of hydrogen bonds between peptide and water was a maximum of three, while that between peptide and number was 20-23. The binding energies decreased to ~ 0 kJ/mol considerably, indicating structural stability **(Rawat *et al.*, 2021).** Initially, the RMSD (Root mean square deviation) of the ligand fluctuated from 2 nm to 8 nm, whereas the rmsd of 5'NT remained stable below 2 nm, whereas after the formation of the complex around 35ns time, the RMSD

became stable. The pressure was approximately 200 bar, and the RMS was between

0.2-0.6 **(Dagar *et al.*,2021)**. The overall simulation analysis showed that gedunin binds to the active centre of 5'NT, and the complex becomes stable. **Figure 4.2.** shows the Ramachandran plot analysis of all complex residues and the analysis using the Prove server with a mean z-score of 0.81. (**Figure 4.3)**

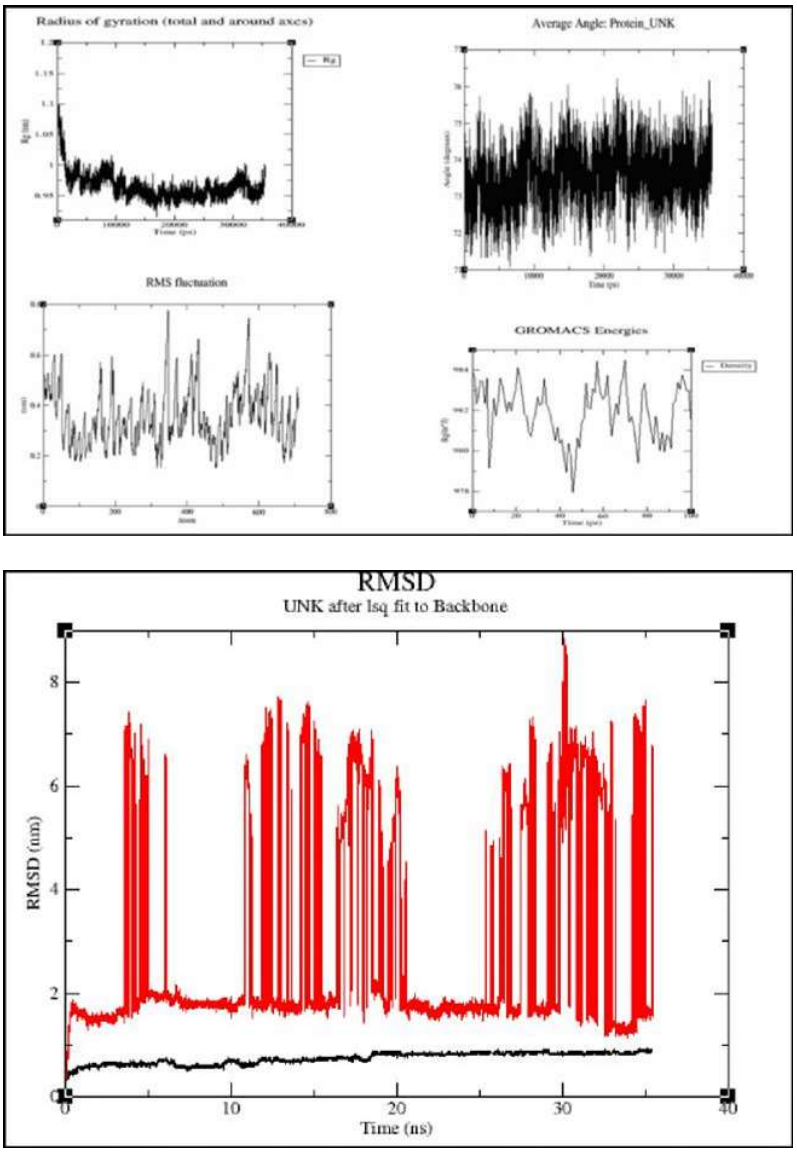

Figure 4.2. 50ns simulation results showing angle, energies, rms, RMSD, and gyration of 5'nt (5'nucleotide) and gedunin. Black represents enzyme 5'nt, whereas red represents the ligand gedunin.

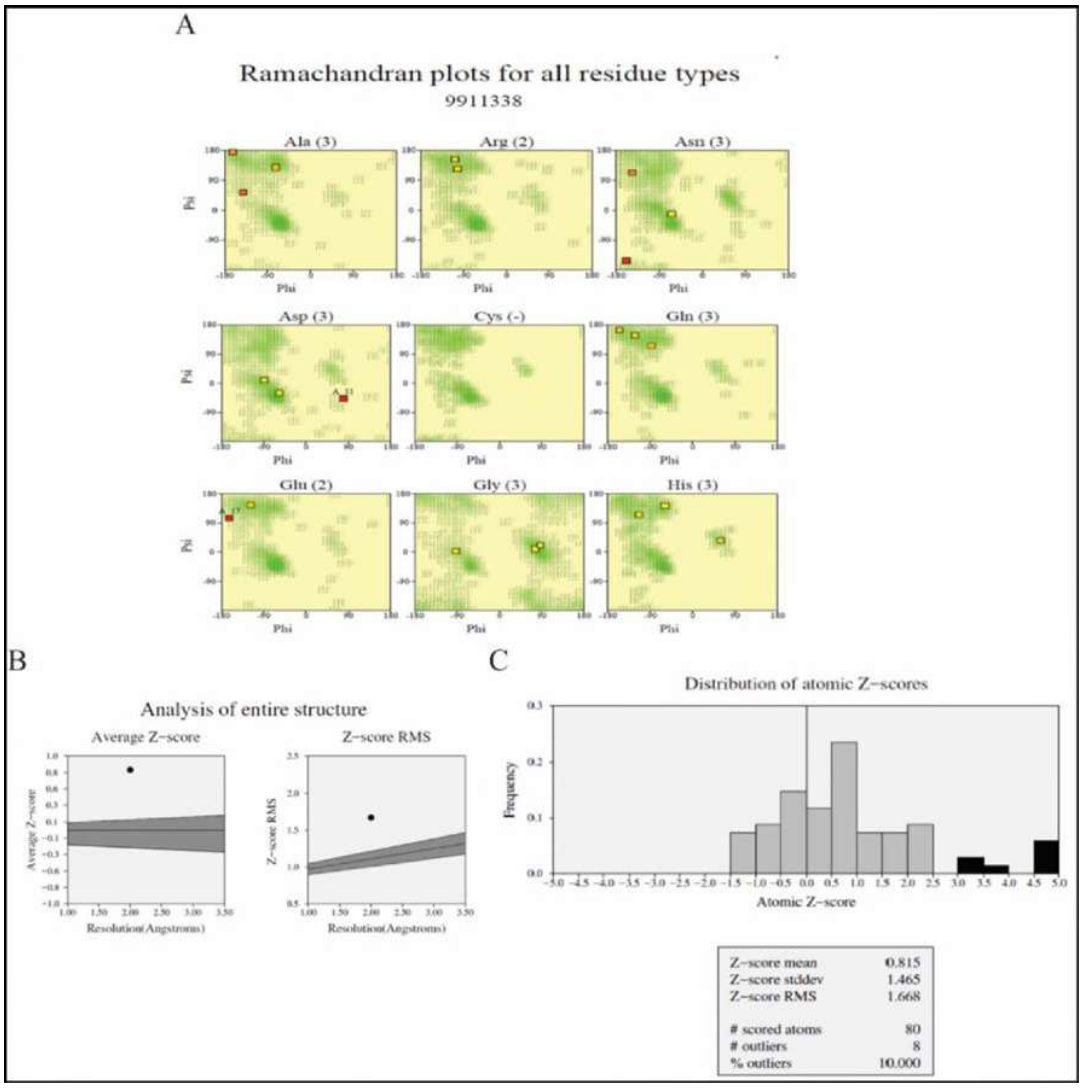

Figure 4.3 Ramachandran plot of all residues of docked complex structure (A) and docked complex structure analysis by the PROVE server (B, C).

4.3.3. Pharmacokinetics

Drug absorption through the GI (Gastrointestinal)tract is measured by human intestinal absorption (HIA), which is responsible for the oral administration of the drug. It is the total absorbed mass/dose of the drug and is reported in **Table 4.3.** for the drug. Lipinski's Rule of Five determines the biological activity of a drug. The Lipinski rule has a molecular weight <500 Dalton (DA), and log P <5 is the

lipophilicity of a compound that can influence many physiological properties, such as metabolic rate and binding site interaction, hydrogen bond acceptor (HBA) <10, and hydrogen bond donor (HBD) <5, all of which can be replaced by physical properties such as TPSA (topological polar surface area), log S> 2 explains the intrinsic solubility of the molecule and is essential in the formulation of any drug molecule. Log D explains the lipophilicity at the relevant pH value and is better suited for predicting the biological activity of the compound**(Shin *et al.*, 2017).**

The blood distribution of the drug makes up the BBB (Blood-brain barrier) category <0.5, the ability of the drug to penetrate the barrier, and is essential in the case of CNS targets, whereas drugs with non-CNS targets and barrier penetration are pretty undesirable. **Table 4.3.** shows that the ligand is a non-CNS drug.

Table 4.3. ADME Properties: The probability scoring profile of gedunin (A) gedunin is Non -CNS and non-BBB permeable and has a low score for 2C9_pKi,(NA is not applicable). (B) the inhibitor is not an inhibitor of p-gp with the most negligible probability score.

A

Profile Name	Score	Standard Deviation	Desired value (According to Lipinski's rule of five)
Intravenous CNS Scoring Profile Score	0.071	0.09	NA
Intravenous Non-CNS Scoring Profile Score	0.175	0.182	NA
Lipinski Rule of Five Score	1	0.002	NA
Oral CNS Scoring Profile Score	0.031	0.070	NA
Oral Non-CNS Scoring Profile Score	0.075	0.142	NA

BBB log brain blood	-0.52	Inf	-0.2 to 1
Log S	0.336	1.033	>2
logS_pH7_4	0.336	1.033	NA
Log P	3.11	0.435	0 -3
Log D	3.11	0.425	0 -3
2C9_pKi	5.331	Inf	<6
hERG_pIC50	4.38	0.914	<6.3
Mol .weight	482.6	0	<500 DA
HBD	0	0	0 – 5
HBA	7	0	0 – 10
TPSA	95.34	0	<140 A^2
Flexibility	0.075	0	NA
Rotatable bonds	3	0	0 -9

B

Property	Category	Probability	Desired value
BBB category	-	0.88	-
HIA category	+	0.50	+
P _gp Inhibitor	No	0.63	NO
2D6_affinity_catego	Very high	0.5625	<6
PPB90_category	Low	0.57	Low

4.3.4. Drug–Likeliness and Medicinal chemistry Using SwissADME

SwissADME, a web-based online server, was used to assess the compound's pharmacokinetics, medicinal chemistry, and drug likelihood. The bioavailability radar considers the drug probability of the ligand:

1. TPSA is the topological polar surface representing the molecule's hydrogen bonding capacity and GI absorption (less than 140 A ^ two good absorption), which determines the blood-brain barrier (less than 90 A ^ two permeable).

2. Lipophilicity (XLOGP3), represented by log P, is suitable for oral bioavailability in the range is 1-3.

3. The solubility (log S) ranged from 1 to 6. The molecular weight and rotational bonds of not more than nine are good in the case of flexibility. The druggability of the molecule is defined using the Lipinski, Veber, Ghosh, and Muegge filters **(Daina *et al.*, 2017).**

The medicinal chemistry region identified potentially problematic fragments. This part has a warning: pain is the molecules containing some substructures that will give a strong response (false positive output) in assays regardless of the protein target. If a molecule contains such units, SwissADME returns warnings. The Brenk filters in SwissADME return warning messages when a reactive, toxic, and metabolically unstable fragment is present. Our ligand has higher Hydrophobicity and size of > 300 and follows Lipinski rule of 5 with no warning signs, thus making it suitable as a "lead" in the lead process optimization process.

4.3.5. Metabolism

Metabolism is the chemical modification of a drug that increases its solubility and hydrophilicity for excretion. P450 isoforms, namely CYP2D6, CYP2C19, CYP2C9, CYP3A4, CYP1A2, CYP2C8, and CYP2E1 metabolizes drugs through oxidation, hydrolysis, and reduction reactions and are present in the liver, gastrointestinal tract, brain, lungs, placenta, and kidney. The cytochrome P450 metabolism model predicted by the Stardrop software has mainly three outputs:

1.P450 predicts which isoforms of the seven cytochrome P450s are the major metabolizing isoforms of a compound. **(Table4.4.)** showed a probability of 0.66 according to isoform 3A4.2.

2. Regioselectivity: This provides information on the most common metabolites that occur when a molecule is an isoform substratum.

0. Localization labiality: This is a measure of the effectiveness of metabolism. C25 and C27 were more labile than the other ligand sites. Inhibition of 2C9_Pki and hERG pic50, shown in **Table 4.4.,** should not be present to avoid drug interactions. 2C9_Pki <6: Predicts the Pki values for CYP2C9 isoform affinity of P450 cytochrome. hERG pic50 <6.3: Inhibition of potassium channel-related gene (hERG) is responsible for a prolonged QT interval, which leads to ventricular arrhythmias. Knowing whether the drug is an inhibitor of this gene is crucial. The ligand showed no inhibition of 2C9_Pki and hERG pic50, as shown in **Table 4.4,** using Star-Drop software.

Table 4.4 P450 Isoform Classification of gedunin using stardrop.

	Isoform Type	Probability value
Majorly belongs to3A4 Isoform	1A2	0.034
	2C19	0.05
	2C8	0.066
	2C9	0.124
	2D6	0.006
	2E1	0.002
	3A4	0.718
P450_3A4_Sites	C1 0.168315 stable C5 1.42164e-6 stable C6 1.27766e-6 stable C7 4.79234e-5 stable C14 0.003791 stable C15 0.003791 stable C16 6.10667e-7 stable C19 1.04296e-7 stable C24 0.000857921 stable	

	C26 1.56387e-6 stable C27 8.85733e-7 stable C28 2.55853e-5 stable C30 0.000444903 stable C31 95.6909 labile C33 4.08356 mod_labile C34 1.62072e-6 stable C35 3.85395e-5 stable C10=C9 0.0520188 stable
P450_3A4_CSL	0.9654
P450_3A4_CSL_Uncertainty	0.0541

4.3.6. Toxicity

The Derek Nexus tool **(Sanderson and Earnshaw, 1991) (Ridings *et al.*, 1996) (Greene *et al.*, 1999)** was used to calculate toxicity risk based on the structural features of the molecule. It is a knowledge-based toxicity prediction tool developed by Lhasa Limited and available on the Stardrop platform **(Optibrium *et al.*, 2021).** A toxicity report indicates the likelihood, such as:

- No report: When there is an endpoint and no reason to raise an alert or predict inactivity or activity based on the molecule's physical properties.
- Equivocal: when there is sufficient and equal evidence for or against the statement.
- Plausible: it is said that the Evidence supports the statement.
- Likely, this is the case when there is no argument against the statement and at least one valid reason for the statement.

The ligand in **Table 4.5** shows the Derek nexus likelihood such that there was an equivocal report in the case of carcinogenicity, whereas it was plausible for skin sensitization, developmental toxicity, hepatotoxicity, skin irritation, and eye irritation.

The toxicity of a drug is due to the groups present in the drug. Biostere **(Review of Biosteres, 2011)** was performed on the ligand to identify the bioactivity of the R groups and their role in ADME properties; Biostere **(Review of Biosteres, 2011)** was performed on the ligand; These are synthetically and chemically validated databases **(Ujvry and Hayward, 2012)** of 29,012 compound pairs that have been combined and cover a wide range of chemical conversions. Biosters are chemical groups with similar chemical and physical properties and have somewhat similar biological properties. If biosters are responsible for the toxicity of a compound, replacing them with another leads to the desired properties without changing the structure of the compound **(ADME, 2021).** Any bioisosteric substitute has the main objective of creating a new, biologically comparable chemical/molecule with the substance in its parent. Bioisosteric replacement is suitable for toxicity mitigation to modify molecular pharmacokinetics. **Figure 4.4** shows the ligand's bioactivity of the R groups using the luminous molecular properties of the Stardrop Software. The blue color shows a decrease in bioactivity, the green color shows no effect, and the red color shows an increase in the group's bioactivity, resulting in an overall change in drug/ligand bioactivity. In the BBB log, nitrogen substitution reduces the predictive value of the permeability of the blood-brain barrier.

Regarding carcinogenicity modifications, the inhibitor did not affect carcinogenicity. Modifications in developmental toxicity did not affect the value of developmental toxicity. Eye irritation modifications did not affect plausible toxicity reports of eye

irritation. Skin irritation modifications did not affect plausible toxicity reports of skin irritation, and HBA modifications increased the number of hydrogen bond acceptors of the inhibitor according to the Lipinski definition. According to the Lipinski definition, HBD modifications increased the number of hydrogen bond donors in the inhibitor. Log P modifications decreased the value of log P. Log S modifications did not affect log S of the inhibitor. Log D modifications decreased the value of log D. TPSA modifications increased the value of TPSA. In the oral CNS, no effect on oral CNS profiling was observed due to the modification of the inhibitor. Oral non-CNS modifications decreased the oral non-CNS profile scores for the inhibitor.

Table 4.5.Toxicity prediction profiling of gedunin using Derek-Nexus Likelihood in Stardrop.Plausible reports support the proposition that gedunin can cause skin and eye irritation. The carcinogenicity profile is equivocal of proposition for and against gedunin.

PROPERTY	REPORT
Photo – allergenicity	No Report
Occupational asthma	No Report
Respiratory sensitization	No Report
Splenotoxicity Probability	No Report
Teratogenicity	No Report
Testicular toxicity	No Report
Adrenal gland toxicity	No Report
Thyroid toxicity	No Report
Ocular toxicity	No Report
Pulmonary toxicity	No Report
Skin sensitization	Plausible
Developmental toxicity	Plausible

Hepatotoxicity	Plausible
Skin irritation	Plausible
Eye irritation	Plausible
Carcinogenicity	Equivocal

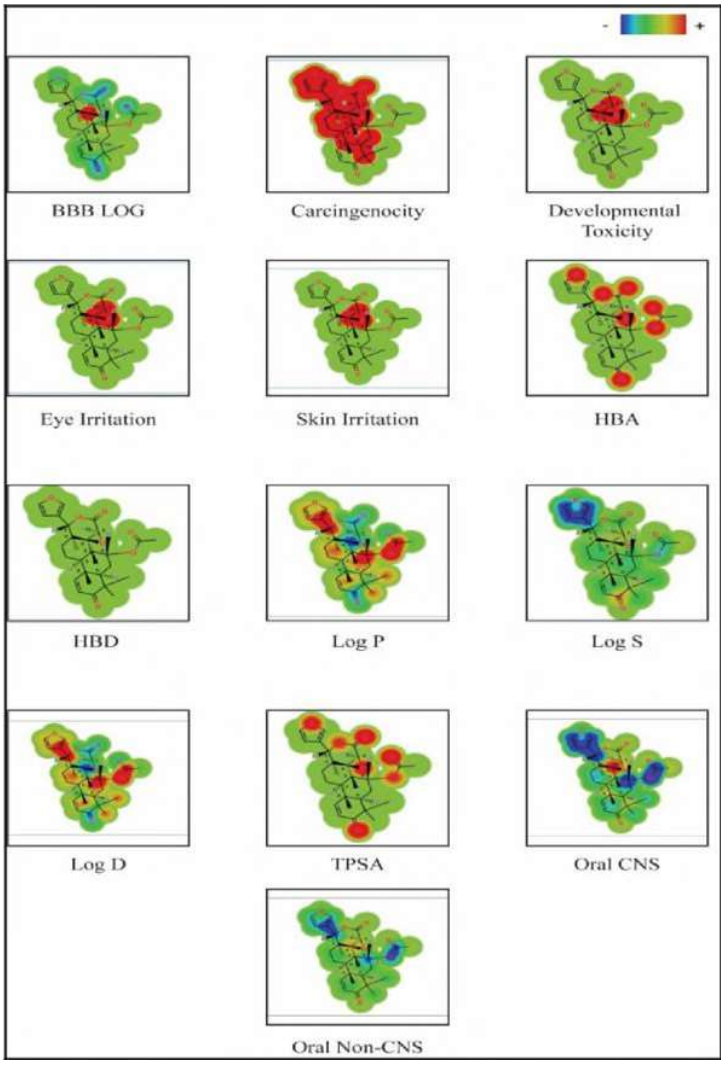

Figure 4.4. Biostere: Glowing molecule visualization of ADMET (ADME + Derek Nexus Likelihood) properties of gedunin. The red region increases the predicted value, and the blue region decreases the predicted value, whereas the green region does not.

4.4. CONCLUSION

Gedunin was identified as a drug candidate based on Lipinski's rule of five. Gedunin's molecular docking and simulation study to act as an inhibitor of the 5'NT snake venom enzyme showed good pharmacokinetic, physicochemical, and drug-like properties. The compound is a P3A4 isoform substrate, showing plausible effects on the skin, eye irritation, sensitization, developmental toxicity, and hepatotoxicity. Modifying the molecule decreased the BBB log, log P, and log D likelihood values while increasing HBA and HBD. A molecule has a reduced chance of crossing the CNS when administered intravenously or orally. The synthesized compound could be used as a therapeutic agent against snake venom. Simulation studies showed that gedunin binds to key enzyme 5'NT (Nucleotidase) at its binding site, and the complex formed was energetically stable.