STM JOURNALS

Research & Reviews: Journal of
Computational Biology

https://journals.stmjournals.com/rrjocb/

ISSN: 2319-3433 (Online)
ISSN: 2349-3720 (Print)
Volume 13, Issue 1, 2024
January–April
DOI (Journal): 10.37591/RRJoCB

Research RRJoCB

In Silico Investigation of 2,3-Dihydrobenzofuran from *Centella asiatica*: A Possible Pioneer in the Management of Tetanus

Harsha*

Abstract

Objective: *Tetanus toxin (TeNT) is a neuroprotein toxin, the most toxic known. They have three functional domains and are structurally similar. They possess an N-terminal catalytic section (light chain), an internal domain for heavy-chain translocation (HN domain), and a C-terminal receptor binding domain for the heavy chain (Hc domain or RBD). Tetanus, an acute nerve-affecting disease, is provoked by a toxin-producing bacterium called Clostridium tetani. This bacterium is environmental, Gram-positive, rod-shaped, spore-forming, and anaerobic. Therefore identifying phytocompounds from the aerial part of Centella asiatica that could potentially be used to treat tetanus is the objective of this study.* ***Methods:*** *An in-silico assessment for tetanus treatment. Based on the aerial part of the plant, which has been marketed as a natural treatment for the condition, the phytocompounds of Centella asiatica have been thoroughly reviewed in this study. Selected compounds (mention compound name) were screened for pharmacology. Finally, using PyRx and Biovia to dock the chosen compound toward the intended protein, the screenings were finished.* ***Results:*** *2,3-Dihydrobenzofuran turned out to be the most effective ligand with the highest negative binding affinity for tetanus treatment.* ***Conclusion:*** *Ideally, Centella asiatica would be suggested as a therapeutic target for future Tetanus in vitro research studies. Centella asiatica mitigates neurological disorders by diminishing inflammatory elements, rectifying aberrant expression of proteins associated with mitochondria, and maintaining equilibrium in oxidative stress levels. The herb is valued in the traditional systems of medicine for the treatment of various chronic disorders such as Alzheimer's diseases, varicose veins, duodenal ulcer, psoriasis, leprosy, certain eczemas, hypertonic scar, and keloids.*

Keywords: *Centella asiatica*, tetanus, phytocompounds, molecular docking, dihydrobenzofuran

INTRODUCTION

The exotoxin secreted by *Clostridium tetani* causes tetanus, an acute and lethal illness. *Clostridium tetani* is an anaerobic, spore-forming, rod-shaped, gram-positive environmental bacterium that produces tetanus toxin (TeNT), a strong neurotoxin that causes spastic paralysis and hyperreflexia [1]. Upon synthesis, TeNT is a single 150 kDa protein that is subsequently broken down into an active toxin form consisting of a heavy chain (HC, 100 kDa) and a light chain (LC, 50 kDa) by an internally occurring protease [2].

***Author for Correspondence**
Harsha
E-mail: harsha722001@gmail.com

Student, Department of Biotechnology, Jain (Deemed-to-be University), Bengaluru, Karnataka, India

Received Date: March 27, 2024
Accepted Date: March 28, 2024
Published Date: April 10, 2024

Citation: Harsha. In Silico Investigation of 2, 3-Dihydrobenzofuran from *Centella Asiatica*: A Possible Pioneer in the Management of Tetanus. Research & Reviews: Journal of Computational Biology. 2024; 13(1): 19–30p.

The immunogenic properties of the known TeNT isoforms remain unchanged from those of prototypical TeNT despite a small number of amino acid variations [3]. TeNT is generally understood to be a protein with three domains, each of which is involved in a distinct stage of neuronal intoxication mechanism [4]. All types of necrotic wounds

(burns, ulcers, surgical wounds, tattoos, circumcisions, needle injections, etc.) can become contaminated by spores that are widely distributed in the environment. These spores can produce vegetative bacteria that produce TeNT, which spreads through blood and lymphatic circulation [5]. TeNT binds to presynaptic nerve terminals in the motor, sensory, and autonomic domains via at least two distinct receptors, a PSG receptor, and a protein receptor [6].

The most significant plants in the medical field are those that have been used for thousands of years to treat human illnesses [7]. They exhibit minimal or no side effects and contain active ingredients with therapeutic value. It grows in some temperate regions of China, Korea, Japan, and Taiwan, as well as in swampy areas of the tropical and subtropical regions of India, Southeast Asia, and Malaysia [8]. The plant, commonly referred to as Indian Pennywort or *Gotu Kola*, is a prized medicinal herb that is used extensively in the East to treat infectious skin conditions and hasten wound and ulcer healing. Various microorganisms can grow and survive in the plant body, which contains a microbiome [9].

The plant possesses anti-inflammatory, antioxidant, antibacterial, antiviral, and anti-rheumatic properties that help to treat venous insufficiency [10]. It also has antipyretic, anti-rheumatic, anxiety-relieving, and anticancer properties. It also has cognitive-enhancing, including neuroprotective, qualities [11]. The primary class of compounds in *C. asiatica* is thought to be pentacyclic triterpenes (PTs), which include madecassoside and asiaticoside as triterpene saponins and their corresponding sapogenins, madecassic acid and asiatic acid, which are referred to as centellosides [12].

An increasing amount of research indicates that *C. asiatica* may have therapeutic benefits in the management of endocrine, neurological, cardiovascular, digestive, respiratory, and dermatological conditions [13]. Several Indian, European, Chinese, and German homeopathic pharmacopoeias list *C. asiatica* as a significant medication [14].

To perform molecular docking against a protein target, 20 phytochemical compounds were extracted from *Centella asiatica*. These compounds have also been evaluated for their ADMET properties. The properties of the best ligands were investigated for protein-ligand docking.

METHODOLOGY

Protein Extraction and Purification

The Protein tetanus neurotoxin [PDB ID:7OH1] was retrieved from the PDB database(https://www.rcsb.org/). It had a resolution of 8.00 Å. It is a macromolecular structure of protein with a 10166 atom count,1285 modelled residue counts with a structural molecular weight of 147.25 kDa.

The protein was purified using Biovia Discovery Studio(https://discover.3ds.com/discovery-studio-visualizer-download). The protein structure was downloaded from the PDB in PDB format. Biovia Discovery Studio uploads the downloaded protein structure. For further analysis, only the A-chain and protein groups were retained in the molecule.

Structure Validation

PDBsum (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) was used to generate a Ramachandran plot of the modelled protein, and the characteristics of the protein were analyzed. It was also used to generate the secondary structure of the modelled protein.

Retrieval of Ligands

Certain phytocompounds of Centella asiatica were downloaded from the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database (*https://cb.imsc.res.in/imppat/basicsearch/phytochemical*). IMPPAT **is** a storehouse for such phytochemicals in medicinal plants in India, depending on their medicinal benefits. Canonical smiles were retrieved from the IMPPAT database.

ADME Analysis

ADME analysis was performed using Swissadme (*http://www.swissadme.ch/*). It describes physiochemical properties such as canonical smiles, formulas, molecular weight, heavy atoms, hydrogen bond acceptors, hydrogen bond donors, and rotatable bonds. Additionally, it provides drug-likeness properties such as the Lipinski rule, Ghose violation, Veber violation, Egan violation, and bioavailability scores. SwissADME was used to generate the boiled eggs.

Molecular Docking

Docking is a technique that forecasts the favored positioning of one molecule to another when a receptor and target are bonded to one another to create a stable combination. Predicting the level of connection or high affinity between the two compounds is possible using an understanding of the random orientation. Molecular docking is one of the most used techniques in configuration pharmaceutical research because it can anticipate the attachment of tiny molecule ligands to the right target receptor complex. Characterization of the binding characteristics is crucial for the functionalization of medications and illuminating basic biological mechanisms. The protein was cleaned using Biovia Discovery Studio by removing water droplets and extra strands that were not needed, followed by the incorporation of proton ions. Subsequently, the refined peptide was subjected to PyRx analysis. It incorporates the editions of the Vina Wizard, Dock Window, and other factors. The use of reducing potency also improves network efficiency. The interaction energy was collected in Csv format after the molecules were tethered to the peptide in Vina Wizard. The compound with the lowest stability constant scores was identified and uploaded to Biovia, where conformational and 2D conformation analyses and predictions of the ligand-protein associations were performed.

RESULTS

Protein Extraction and Purification

Extraction of the protein of interest is required to characterize its shape, interconnections, and activity. During purification, the heteroatom constituents must first be separated. Extra strands can influence binding results and add complications to the target protein, but they are not necessary for the docking process. As a result, before binding, Biovia filters render it clean and add polarity charges. In the Figure 1 (a) Unpurified target protein is shown in Figure 1(b).

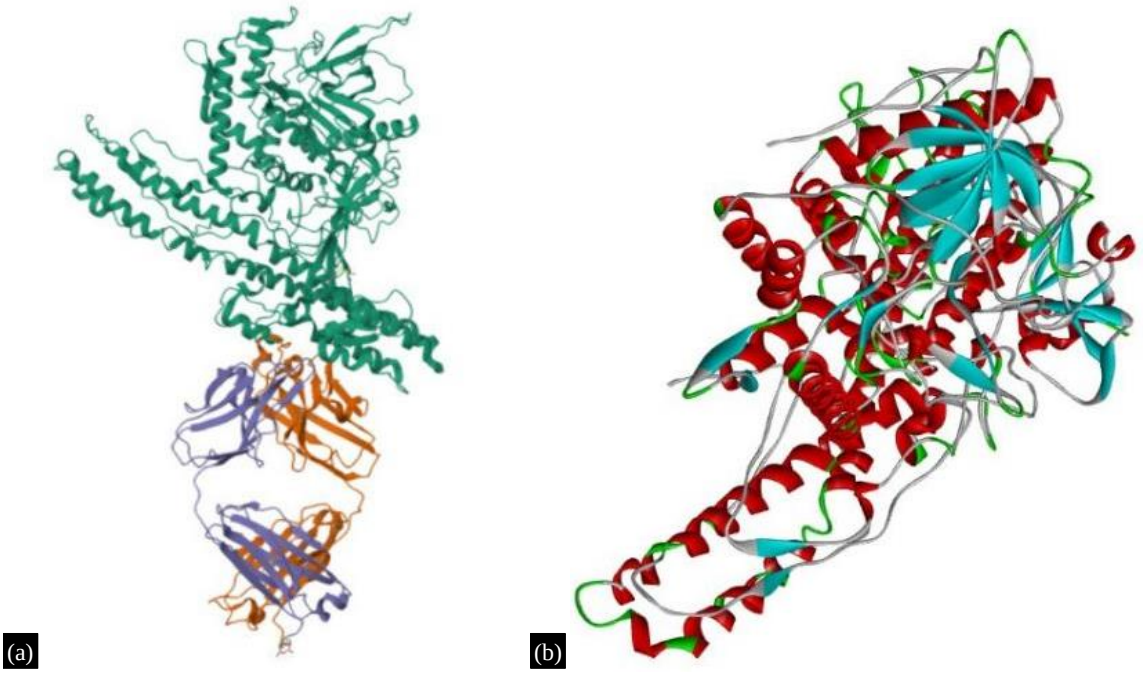

Figure 1. (a) Unpurified target protein and (b)Purified target protein.

Structure Validation

A Ramachandran plot was used to show the descriptive statistical dispersion of both the phi and psi inclinations for every peptide in the molecule.

The Ramachandran plot showed that 86.5% of the areas had the most favored regions. Additional allowed regions (13.1%) Generously allowed regions of 0.4%. Disallowed region: 0.0%. Non-glycine and non-proline residues, 100%. A Ramachandran plot of the target proteins is shown in Figure 2.

The secondary structure of the target protein generated from PDBsum has 11 sheets,1 beta alpha beta units,8 beta hairpins,1 psi loops,4 beta bulges,30 strands,30 helices,48 helix-helix interactions,85 beta turns,10 gamma turns,1 and disulfide, as shown in Figure 3.

Retrieval of Ligands

The aerial parts of *Centella asiatica's* aerial part and canonical smiles were retrieved from the PubChem database shown in Table 1.

ADME Analysis

We have compared the physiochemical properties, such as molecular weight (MW); the acceptable range of molecular weight must not be more than 500 Da. The hydrogen bond acceptors (HBA) range must be less than or equal to 10. The hydrogen bond donor (HBD) range must be less than or equal to five. The number of rotatable bonds (RA) represents the stability of the bioactive substances. Fsp^3 is the fraction of carbon atoms that are sp^3 hybridized, topological polar surface area (TPSA), number of charged groups, total charge of the compound, number of carbons, hetero- and heavy atoms, and the ratio of the number of non-carbon atoms to the number of carbon atoms, as shown in Table 2.

Crucial elements such as GIA (Gastro intestinal) absorption should be considered during drug discovery. The substratum for permeability glycoproteins (PGP) helps the secretion mechanism to move through the cell. Log Kp (skin permeation) facilitates the cutaneous absorption of medication. The pharmacokinetic properties of the phytocompounds extracted from *Centella asiatica* are shown in Table 3.

Drug-likeness is the qualitative description of the oral bioavailability of a therapeutic compound. This evaluation is critical because many drugs are orally administered. The acceptable range of molecular weights must not exceed 500 Da. The hydrogen bond acceptors (HBA) range must be less than or equal to 10. The hydrogen bond donor (HBD) range must be less than or equal to five. Table 4 shows the drug-likeness properties of the phytocompounds extracted from *Centella asiatica*

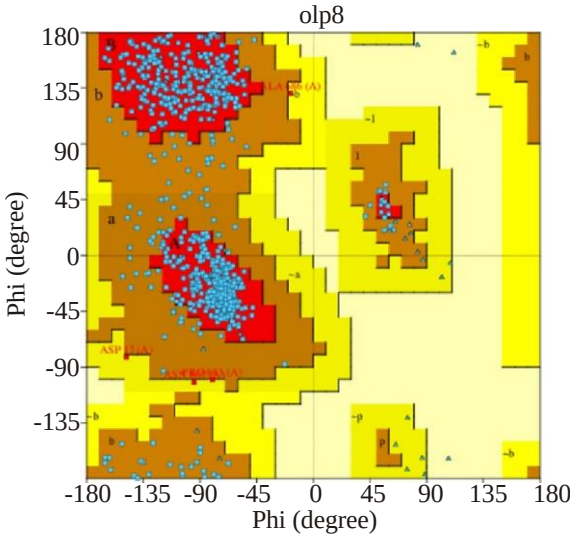

Figure 2. Ramachandran plot of the target protein.

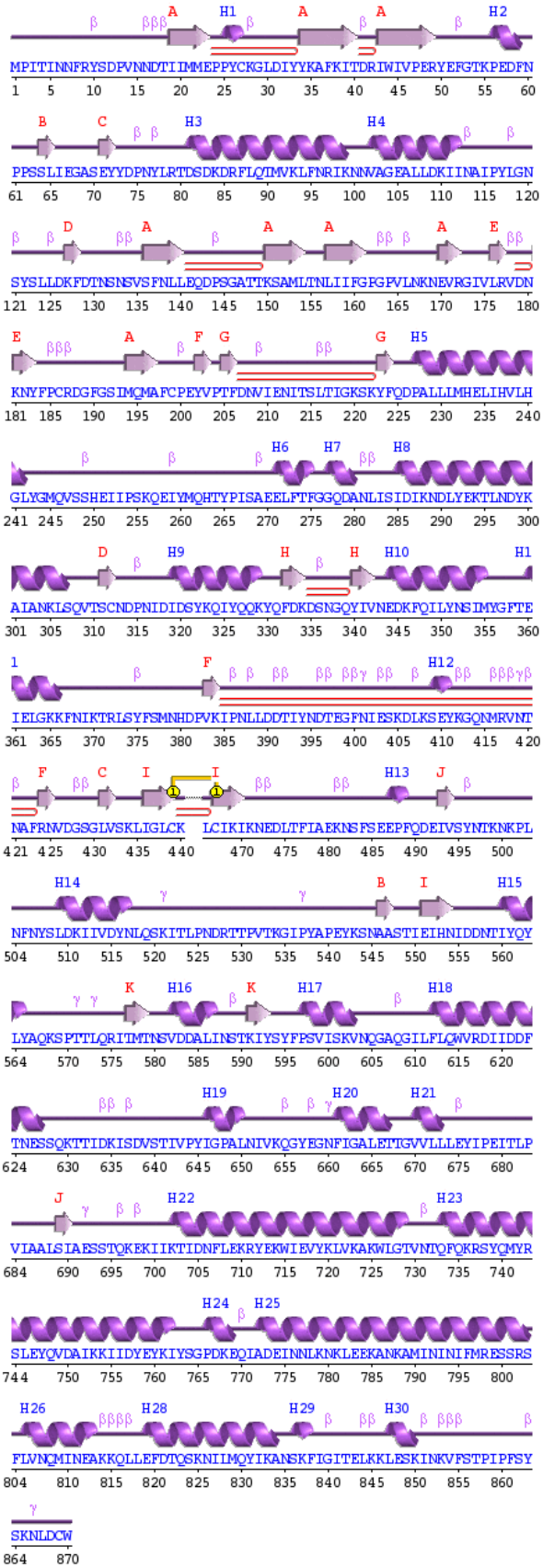

Figure 3. Secondary Structure of the protein 7OH1. The secondary structure of target protein generated from PDBsum.

Table 1. Phytocompounds of *Centella asiatica's* aerial part

S.N.	Ligand Name	Canonical Smiles
01.	beta-Bisabolene	CC1=CCC(CC1)C(=C)CCC=C(C)C
02.	2,3-Dihydrobenzofuran	C1COC2=CC=CC=C21
03.	Myrcene	CC(=CCCC(=C)C=C)C
04.	2-Methoxy-4-vinylphenol	COC1=C(C=CC(=C1)C=C)O
05.	2-Heptenal	CCCCC=CC=O
06.	gamma-Terpinene	CC1=CCC(=CC1)C(C)C
07.	2-Octenal	CCCCCC=CC=O
08.	Hexanal	CCCCCC=O
09.	2-Methyl-2-butanol	CCC(C)(C)O
10.	Corosolic acid	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CC(C(C5(C)C)O)O)C)C)C2C1C)C)C(=O)O
11.	m-Xylene	CC1=CC(=CC=C1)C
12.	Furfural	C1=COC(=C1)C=O
13.	Heptanal	CCCCCCC=O
14.	Pentanal	CCCCC=O
15.	(-)-beta-Chamigrene	CC1=CCC2(CC1)C(=C)CCCC2(C)C
16.	Toluene	CC1=CC=CC=C1
17.	(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	CC1=CCCC(=C)C=CC(CC1)C(C)C
18.	Farnesol	CC(=CCCC(=CCCC(=CCO)C)C)C
19.	beta-Farnesene	CC(=CCCC(=CCCC(=C)C=C)C)C
20.	Humulene	CC1=CCC(C=CCC(=CCC1)C)(C)C

Table 2. Physico chemical properties of phytocompounds extracted from *Centella asiatica*

Molecule Name	Formula	MW	HA	AHA	Fraction CSP3	RB	HBA	HBD	MR	TPSA
beta-Bisabolene	C15H24	204.35	15	0	0.6	4	0	0	70.68	0
2,3-Dihydrobenzofuran	C8H8O	120.15	9	6	0.25	0	1	0	35.79	9.23
Myrcene	C10H16	136.23	10	0	0.4	4	0	0	48.76	0
2-Methoxy-4-vinylphenol	C9H10O2	150.17	11	6	0.11	2	2	1	45.05	29.46
2-Heptenal	C7H12O	112.17	8	0	0.57	4	1	0	35.49	17.07
gamma-Terpinene	C10H16	136.23	10	0	0.6	1	0	0	47.12	0
2-Octenal	C8H14O	126.2	9	0	0.62	5	1	0	40.3	17.07
Hexanal	C6H12O	100.16	7	0	0.83	4	1	0	31.16	17.07
2-Methyl-2-butanol	C5H12O	88.15	6	0	1	1	1	1	27.35	20.23
Corosolic acid	C30H48O4	472.7	34	0	0.9	1	4	3	138.08	77.76
m-Xylene	C8H10	106.17	8	6	0.25	0	0	0	36.37	0
Furfural	C5H4O2	96.08	7	5	0	1	2	0	24.1	30.21
Heptanal	C7H14O	114.19	8	0	0.86	5	1	0	35.96	17.07
Pentanal	C5H10O	86.13	6	0	0.8	3	1	0	26.35	17.07
(-)-beta-Chamigrene	C15H24	204.35	15	0	0.73	0	0	0	68.52	0
Toluene	C7H8	92.14	7	6	0.14	0	0	0	31.41	0
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	C15H24	204.35	15	0	0.6	1	0	0	70.68	0
Farnesol	C15H26O	222.37	16	0	0.6	7	1	1	73.96	20.23
beta-Farnesene	C15H24	204.35	15	0	0.47	7	0	0	72.32	0
Humulene	C15H24	204.35	15	0	0.6	0	0	0	70.42	0

Table 3. Pharmacokinetics properties of phytocompounds extracted from *Centella asiatica.*

Molecule Name	GI Absorption	BBB Permeant	P-gp Substrate	Log Kp (skin permeation)
beta-Bisabolene	Low	No	No	-2.98
2,3-Dihydrobenzofuran	High	Yes	No	-5.51
Myrcene	Low	Yes	No	-4.17
2-Methoxy-4-vinylphenol	High	Yes	No	-5.22
2-Heptenal	High	Yes	No	-5.52
gamma-Terpinene	Low	Yes	No	-3.94
2-Octenal	High	Yes	No	-5.22
Hexanal	High	Yes	No	-5.65
2-Methyl-2-butanol	High	Yes	No	-6.21
Corosolic acid	High	No	Yes	-4.66
m-Xylene	Low	Yes	No	-4.68
Furfural	High	Yes	No	-6.6
Heptanal	High	Yes	No	-5.35
Pentanal	High	Yes	No	-6.05
(-)-beta-Chamigrene	Low	No	No	-4.23
Toluene	Low	No	No	-4.92
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	Low	No	No	-4.18
Farnesol	High	Yes	No	-3.81
beta-Farnesene	Low	No	No	-3.27
Humulene	Low	No	No	-4.32

Table 4. Druglikeness properties of phytocompounds extracted from *Centella asiatica.*

Molecule Name	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
beta-Bisabolene	Yes	Yes	Yes	Yes	No	0.55
2,3-Dihydrobenzofuran	Yes	No	Yes	Yes	No	0.55
Myrcene	Yes	No	Yes	Yes	No	0.55
2-Methoxy-4-vinylphenol	Yes	No	Yes	Yes	No	0.55
2-Heptenal	Yes	No	Yes	Yes	No	0.55
gamma-Terpinene	Yes	No	Yes	Yes	No	0.55
2-Octenal	Yes	No	Yes	Yes	No	0.55
Hexanal	Yes	No	Yes	Yes	No	0.55
2-Methyl-2-butanol	Yes	No	Yes	Yes	No	0.55
Corosolic acid	Yes	No	Yes	**No**	No	0.56
m-Xylene	Yes	No	Yes	Yes	No	0.55
Furfural	Yes	No	Yes	Yes	No	0.55
Heptanal	Yes	No	Yes	Yes	No	0.55
Pentanal	Yes	No	Yes	Yes	No	0.55
(-)-beta-Chamigrene	Yes	Yes	Yes	Yes	No	0.55
Toluene	Yes	No	Yes	Yes	No	0.55
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	Yes	Yes	Yes	Yes	No	0.55
Farnesol	Yes	Yes	Yes	Yes	No	0.55
beta-Farnesene	Yes	Yes	Yes	Yes	No	0.55
Humulene	Yes	Yes	Yes	Yes	No	0.55

Boiled Egg Analysis

The Central Nervous System or Gastrointestinal Estimated Permeability Prediction Model predicts the GI absorption capacity of the pharmaceutical component, which is a vital factor in the development and design of medications (BOILED-Egg). Ligand compounds that drop in the BOILED-white Egg's portion are predicted to be more likely to be absorbed by the GI tract, whereas those that drop in the yellow areas are predicted to pass through the BBB. PGP is present in the compounds, as shown by a blue circle point, whereas PGP is absent, as indicated by a red circle point. Figure 4 shows the analysis based on boiled eggs.

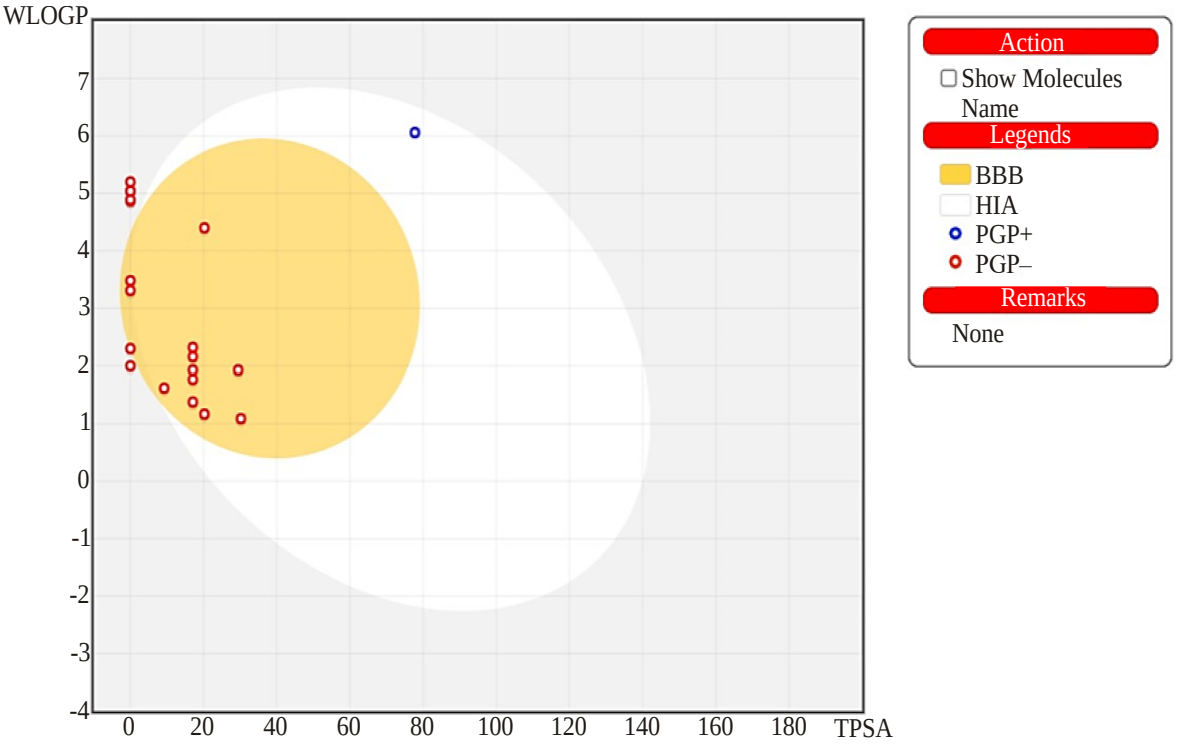

Figure 4. Boiled egg analysis.

Molecular Docking and Visualization

The selected compound 2,3-Dihydrobenzofuran, was docked with a clean protein using the Biovia software. The highest negative binding energy of the ligand was obtained for visualization. In Table 5, the binding energy of the ligands with protein 7OH1 is shown in Figure 5 (a), which shows the 2,3-Dihydrobenzofuran binding with the ligand, and in Figure 5 (b), we can see the secondary structure of 2,3-Dihydrobenzofuran docked with clean protein.

Table 5. Binding affinity of the targeted protein 7OH1and ligands from *centella asiatica*

Compound Name	Binding Affinity
7OH1_2,3-Dihydrobenzofuran	-5.9
7OH1_2-Methoxy-4-vinylphenol	-5.7
7OH1_gamma-Terpinene	-5.7
7OH1_m-Xylene	-5.5
7OH1_Myrcene	-4.8
7OH1_2-Heptenal	-4.7
7OH1_2-Octenal	-4.7
7OH1_2-Methyl-2-butanol	-4.2
7OH1_Hexanal	-3.9

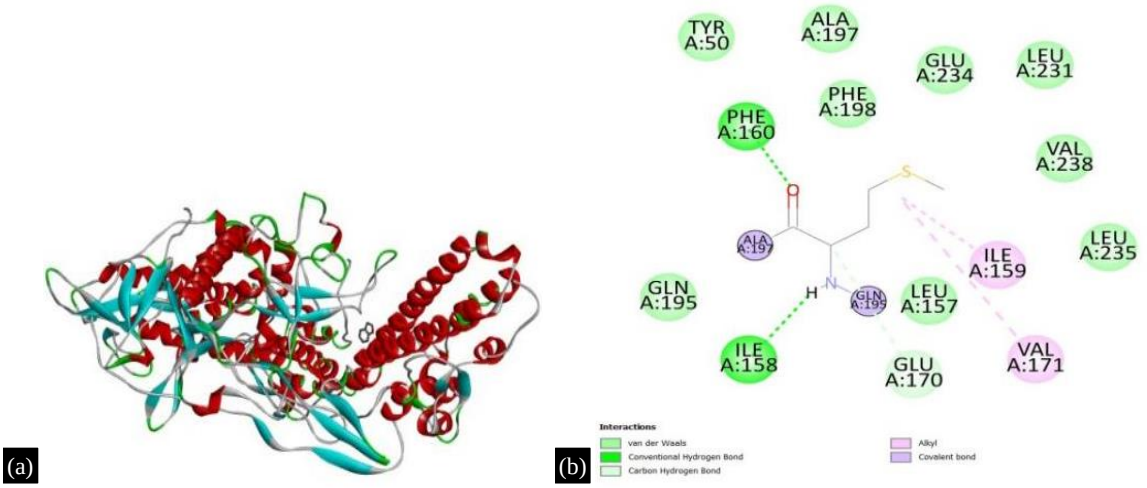

Figure 5. (a) 2,3-Dihydrobenzofuran docked with clean protein and (b) Secondary structure of 2,3-Dihydrobenzofuran docked with clean protein.

DISSCUSSION

Infection occurs when spores enter the body through the skin or mucous membrane breaks; it is not passed from person to person.

TeNT interacts with demyelinated nerve endings by diffusing locally at the infection site [15]. TeNT identifies particular receptors on the membranes of neurons that are composed of a membrane protein and ganglioside component [16]. At the neuromuscular junction, TeNT identifies the extracellular matrix protein nidogen [17]. TeNTs internalized through receptor-mediated endocytosis bind to their receptors. Following this, the toxin is directed toward non-acidified endocytic vesicles [18]. The main causes of death in tetanus patients are respiratory failure, central nervous system disruption, and inhibition of neurotransmitter release, all of which can be induced by TeNT [19].

Centella asiatica is a medicinal herb belonging to the Apiaceae (umbelliferae) family. Its numerous names include *Gotu Kola, luei gong gen,* Indian pennywort, *pegaga, pegagan, mandukaparni,* and *ab-boshghabi* [20]. Edible green, slender leaves with seven elliptical forms and long petiole alternates are produced by *C. asiatica* [21]. Green to red stolons that merge with underground roots allow the plants to grow horizontally. Vegetatively and sexually, they can be produced easily. After three–four months of cultivation, plants can be grown without the need for special care [22]. Treatments for numerous medical conditions have been employed for *Centella asiatica*. It is also consumed as a leafy green vegetable with a flavor that is both aromatic and slightly spicy [23].

They are primarily responsible for the biological activities of C. asiatica. As it eliminates free radicals and has antimicrobial properties against a range of microorganisms, C. asiatica extract has excellent antioxidant properties [24]. In addition, the plant has anti-inflammatory, hepatoprotective, antinociceptive, antidiabetic, and wound-healing properties. It is now marketed as a liposomal tablet, powder, or cream, and is a crucial part of over a hundred traditional and modern [25].

Their efficacy against tetanus neurotoxin was examined using phytocompounds derived from *Centella asiatica* as a source. Their interactions were investigated in silico by docking the 2,3-Dihydrobenzofuran compound into the tetanus target protein.

CONCLUSION

Centella asiatica has numerous medical benefits, such as anti-inflammatory, antioxidant, antibacterial, and antiviral properties that help treat venous insufficiency; anti-rheumatic, antipyretic, anxiety-relieving, and anti-cancer properties; and cognitive-enhancing, including neuroprotective

properties. Research suggests that Centella asiatica can be used as a strong antibacterial agent to treat tetanus. Twenty phytocompounds were used in silico, and 2,3-Dihydrobenzofuran was the binder with the highest binding affinity.

They can be used to strengthen the pharmacological interventions intended to treat tetanus. Analyzing the therapeutic properties of related plants with built-in antioxidants for tetanus prevention is another possibility.

Acknowledgement

Thus, I thank BioNome, Bengaluru, India's Department of Bioinformatics, for offering computing resources and assistance with empirical research. I appreciate Miss Samiksha Bhor's help with this project.

Abbreviations

AHA—Aromatic Heavy Atoms

HIA—Human Intestinal Absorption

Log kp—Skin permeation

PDB—Protein Data Bank

P-GP—Permeability Glycoprotein

IMPPAT—Indian Medicinal Plants, Phytochemistry and Therapeutics

TeNT—Tetanus Neurotoxin

ADME—Absorption, Distribution, Metabolism,Excretion

C.asiatica—Centella asiatica

BBB—Blood Brain Barrier

HBA—Hydrogen Bond Acceptors

HBD—Hydrogen Bond Donors

MR—Molar refractivity

MW—Molecular Weight

RB—Rotatable Bonds

TPSA—Topological Polar Surface Area

REFERENCES

1. Megighian A, Pirazzini M, Fabris F, Rossetto O, Montecucco C. Tetanus and tetanus neurotoxin: From peripheral uptake to central nervous tissue targets. J Neurochem. 2021;158:1244–1253. DOI: 10.1111/jnc.15330. PubMed: 33629408.
2. Francotte A, Esson R, Abachin E, Vanhamme M, Dobly A, Carpick B, Uhlrich S, Dierick JF, Vanhee C. Development and validation of a targeted LC-MS/MS quantitation method to monitor cell culture expression of tetanus neurotoxin during vaccine production. Talanta. 2022;236:122883. DOI: 10.1016/j.talanta.2021.122883. PubMed: 34635263.
3. Kandasamy A, Aruchamy K, Rangasamy P, Varadhaiyan D, Gowri C, Oh TH, Ramasundaram S, Athinarayanan B. Phytochemical analysis and antioxidant activity of Centella asiatica extracts: An experimental and theoretical investigation of flavonoids. Plants. 2023;12. DOI: 10.3390/plants12203547. PubMed: 37896010.
4. Pirazzini M, Montecucco C, Rossetto O. Toxicology and pharmacology of botulinum and tetanus neurotoxins: An update. Arch Toxicol [Internet]. 2022;96:1521–1539. DOI: 10.1007/s00204-022-03271-9. PubMed: 35333944.
5. Pratama RA, Astina J, Parikesit AA. In silico screening of potential antidiabetic phenolic compounds from banana (Musa spp.) peel against PTP1B protein. J Trop Biodivers Biotechnol. 2023;8. DOI: 10.22146/jtbb.83124.

6. Popoff MR. Tetanus in animals. J Vet Diagn Invest. 2020;32:184–191. DOI: 10.1177/1040638720906814. PubMed: 32070229.
7. Malinovská Z, Čonková E, Váczi P. Tetanus in animals — Summary of knowledge. Folia Veterinaria. 2020;64:54–60. DOI: 10.2478/fv-2020-0027.
8. Pirazzini M, Grinzato A, Corti D, Barbieri S, Leka O, Vallese F, et al. Extremely potent human monoclonal antibodies for the prophylaxis and therapy of tetanus. bioRxiv. DOI: 10.1101/2021.05.24.445390.
9. Zhang Y, Yang Z, Cock IE. Centella asiatica (L.) urban leaf extracts inhibit the growth of bacterial triggers of selected autoimmune inflammatory diseases and potentiate the activity of conventional antibiotics. Phcog Commun. 2020;10:119–129. DOI: 10.5530/pc.2020.3.24.
10. Dhiman S, Nadda RK, Bhardwaj P. Medicinal herbs from Western Himalayas for hemorrhoids treatment: A review correlating traditional knowledge with modern therapeutics. Pharmacol Res Mod Chin Med. 2023.
11. Hodgins HP, Chen P, Lobb B, Wei X, Tremblay BJM, Mansfield MJ, Lee VCY, Lee PG, Coffin J, Duggan AT, Dolphin AE, Renaud G, Dong M, Doxey AC. Ancient Clostridium DNA and variants of tetanus neurotoxins associated with human archaeological remains. Nat Commun. 2023;14:5475. DOI: 10.1038/s41467-023-41174-0. PubMed: 37673908.
12. Nouri Nav S, Nejad Ebrahimi S, Sonboli A, Mirjalili MH. Variability, association and path analysis of centellosides and agro-morphological characteristics in Iranian Centella asiatica (L.) Urban ecotypes. S Afr J Bot. 2021;139:254–266. DOI: 10.1016/j.sajb.2021.03.006.
13. Hasan R, Rony MNH, Ahmed R. In silico characterization and structural modeling of bacterial metalloprotease of family M4. J Genet Eng Biotechnol. 2021;19:25. DOI: 10.1186/s43141-020-00105-y. PubMed: 33528696.
14. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, Kudo M, Gao M, Liu T. Therapeutic potential of Centella asiatica and its triterpenes: A review. Front Pharmacol. 2020;11:568032. DOI: 10.3389/fphar.2020.568032. PubMed: 33013406.
15. Li BL, Wang JR, Liu XY, Lu JS, Wang R, Du P, Yu S, Pang XB, Yu YZ, Yang ZX. Tetanus toxin and botulinum neurotoxin-derived fusion molecules are effective bivalent vaccines. Appl Microbiol Biotechnol. 2023;107:7197–7211. DOI: 10.1007/s00253-023-12796-7. PubMed: 37741939.
16. Cai S, Kumar R, Singh BR. Clostridial neurotoxins: Structure, function and implications to other bacterial toxins. Microorganisms. 2021;9:2206. DOI: 10.3390/microorganisms9112206. PubMed: 34835332.
17. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P, Samal A. IMPPAT: A curated database of Indian medicinal plants, phytochemistry and therapeutics. Sci Rep. 2018;8. DOI: 10.1038/s41598-018-22631-z.
18. Fuad FM, Nadzir MM. Ultrasound-assisted extraction of asiaticoside from Centella asiatica using betaine-based natural deep eutectic solvent. Ind Crops Prod. 2023;192.
19. Tripathy S, Verma DK, Thakur M, Chakravorty N, Singh S, Srivastav PP. Recent trends in extraction, identification and quantification methods of Centella asiatica phytochemicals with potential applications in food industry and therapeutic relevance: A review. Food Biosci. 2022;49:101864. DOI: 10.1016/j.fbio.2022.101864.
20. Mir WR, Bhat BA, Rather MA, Muzamil S, Almilaibary A, Alkhanani M, Mir MA. Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of Geranium wallichianum D. Don ex Sweet from Kashmir Himalaya. Sci Rep. 2022;12:12547. DOI: 10.1038/s41598-022-16102-9. PubMed: 35869098.
21. Tan SC, Bhattamisra SK, Chellappan DK, Candasamy M. Actions and therapeutic potential of madecassoside and other major constituents of Centella asiatica: A review. Appl Sci (Basel). 2021;11:8475. DOI: 10.3390/app11188475.
22. Arribas-López E, Zand N, Ojo O, Snowden MJ, Kochhar T. A systematic review of the effect of Centella asiatica on wound healing. Int J Environ Res Public Health. 2022;19:3266. DOI: 10.3390/ijerph19063266. PubMed: 35328954.

23. Singh R, Kharsyntiew B, Sharma P, Sahoo UK, Sarangi PK, Prus P, Imbrea F. The effect of production and post-harvest processing practices on quality attributes in Centella asiatica (L.) Urban—A review. Agronomy. 2023;13. DOI: 10.3390/agronomy13081999.
24. Seong E, Heo H, Sang Jeong H, Lee H, Lee J. Enhancement of bioactive compounds and biological activities of Centella asiatica through ultrasound treatment. Ultrason Sonochem. 2023;94:106353. DOI: 10.1016/j.ultsonch.2023.106353. PubMed: 36889177.
25. Prasad A, Mathur AK, Mathur A. Advances and emerging research trends for modulation of centelloside biosynthesis in Centella asiatica (L.) Urban—A review. Ind Crops Prod. 2019;141. DOI: 10.1016/j.indcrop.2019.111768.

