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Computational insights of *Catharanthus roseus* phytochemicals against putative proteins of pathogenic *Yersinia ruckeri* to combat red mouth disease in salmonid fishes

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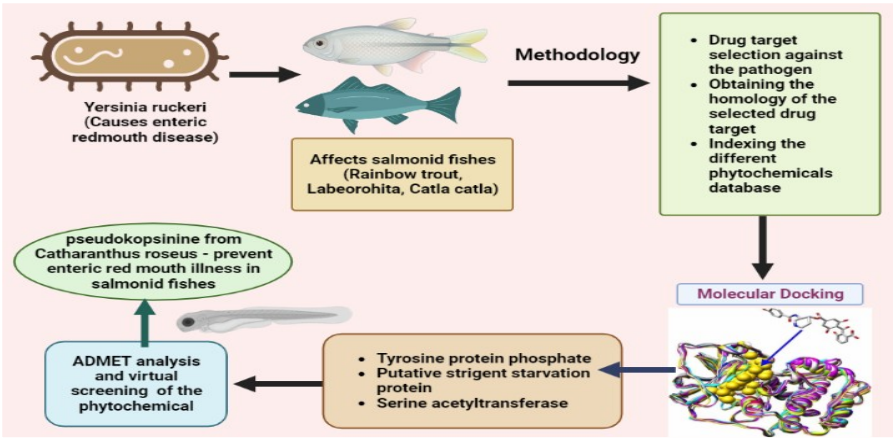
Article

Abstract



Yersinia ruckeri is a Gram-negative rod-shaped bacterium that causes enteric red mouth disease explicitly in salmonid fishes such as Rainbow trout and other common fishes like *Labeo rohita* and *Catla catla*. This disease is proven to increase the mortality rates of infected fish, which has many significant health benefits for human beings. Indian ethno-botanical plant like *Catharanthus roseus* are rich in secondary metabolites and phytochemicals. Bioinformatics approach is applied in our study to find therapeutic agents that could inhibit the pathogenic proteins in the causative agent. Docking techniques have deciphered that the phytochemical Pseudokopsinine originating from the plant *Catharanthus roseus* shows a significant inhibition potential of -9.4 kcal/mol, -10.6 kcal/mol, -11 kcal/mol with the proteins, Tyrosine-protein phosphatase YopH (PDB ID-1LYV), Putative stringent starvation protein A (PDB ID-1YY7) and Serine acetyltransferase respectively. Pseudokopsinine fulfils practically all of the factors taken into account in ADME/T analysis. Therefore, this *insilico* phytochemical approach could be utilized as an aid to prevent the enteric red mouth disease in the salmonid fishes.

Keywords: Catharanthus roseus, Yersinia ruckeri, ADME/T, Phytochemicals, Molecular Docking, Pseudokopsinine

INTRODUCTION

Yersinia ruckeri belongs to the Enterobacteriaceae family and is a Gram-negative, rod-shaped bacteria that generally possess rounded ends. It is a facultatively anaerobic microscopic organism. These are actively growing cells, and the size varies from 1 to 3 micrometers in length and 0.75 micrometers in diameter.[1] These are primarily flagellar and non-spore-forming bacterium that lacks a true capsule. *Yersinia ruckeri* can also be recognized by its property of fermenting glucose and mannitol and negative oxidase and nitrate reduction test. This species of *Yersinia* can be distinguished from other strains due to the presence of lysine decarboxylase, beta-galactosidase, and ornithine decarboxylase. They also indicate a negative indole test and doesn't exhibit the production of H_2S gas. It is the causative agent of enteric red mouth disease, primarily affecting the salmonid fish species.[2] This bacterial species is responsible for causing significant economic losses in salmonid aquaculture throughout the world. Even though this species is responsible for causing a very substantial disease among the aquatic population, not much information has been found about the mechanism of its pathogenesis.

Yersiniosis, or enteric red mouth disease, was first isolated in rainbow trout or *Oncorhynchus mykiss*.[1] It is responsible for increasing the mortality rates and causing economic losses in culturing salmonid fishes. *Yersinia ruckeri* consists of five outer membrane proteins: OMP 1, OMP 2, OMP 3, OMP 4, and OMP 5. It also includes five O- serotypes from O1 to O5 and type 1 and 2 biotypes.[3] To date, almost 20 different species of salmonid fish have been known to be affected by this bacterium. There is a possibility of other species being infected by this disease, which leads to decreased aquaculture trade. The most common symptoms shown by fishes include inactive behavior,[4,5] swimming near the surface of the water[6], darkening of the skin,[7] and lack of appetite or anorexia. Other diverse effects include

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reddening the mouth and throat, followed by subcutaneous hemorrhage among the affected fishes.[8] Some of the host species affected by *Yersinia ruckeri* other than rainbow trout include Cod, Goldfish, Eel, and African Catfish.[3]

Among the infected species, Rainbow trout is the most useful for mankind. This is so because extensive research is being carried out in toxicology, carcinogenesis, immunology, nutrition, and physiology.[9] Cod liver oil is rich in Vitamin A and is used in making supplements for people who suffer from Vitamin A deficiency.[10] Diseases in such species cause several indirect harmful effects on humans as well. Mankind needs to find a cure for the enteric red mouth to protect the fish and, indirectly, humanity. Computational biology when linked with bioinformatics provides us the scope and source of finding possible drugs obtained from Indian ethnobotanical plants, known to be rich in secondary metabolites and phytochemicals, for the treatment of Enteric Red mouth diseases in the salmonid fishes, proven to have lesser side effects with respect to other synthetic chemicals.[11]

Catharanthus roseus, known as Madagascar periwinkle, is rich in alkaloids.[12] It is also said to be rich in both enzymatic and non-enzymatic antioxidants. The alkaloids derived from *C. roseus* are potent sedative, considered to be hypotensive, and have a high tranquilizing effect. It is proven to relieve muscle spasms and pain and is effective against wasp stings. It can also help cure depression in the Central Nervous System. Not only depression, but it can also treat hypertension, memory loss, cystitis, diarrhea and hypoglycaemia. It is said to increase sugar utilization from the body's food and enhance insulin production. In daily life, it can treat bleeding gum, nose bleeds, sore throats, and mouth ulcer issues. It has also proven helpful in cancer treatment.[13] Several studies have been conducted previously to determine the antibacterial effects of this plant. The crude extracts from several *C. roseus* sections have been tested against clinically significant bacterial strains to establish their antibacterial efficacy.[14]

A disc diffusion assay was conducted as part of the experiment to determine the antibacterial activity of the compounds against certain bacterial strains after the bioactive components had been extracted in the appropriate solvents. The data show that the extraction method, plant component, physiological and morphological state of the plant, extraction solvent, and microorganism under inquiry all had a substantial influence on the inhibitory pattern. Antibacterial activity was more prominent in the organic solvent than in aqueous solvent extracts.[14] However, there was no sign of action in the aqueous extracts. The ethanol extract demonstrated the highest efficacy against the tested bacterial strains among the significantly active extracts. More sensitive than Gram (+) microorganisms were Gram (-) stains. According to such studies, *C. roseus* bioactive compound(s) may be applied as antibiotics.[14]

The study's primary objectives are to understand *Yersinia ruckeri*, the causative agent of enteric red mouth disease in salmonid fish, investigate its impact, and explore potential treatments. It aims to assess the antibacterial properties of *Catharanthus roseus* extracts and their potential as natural antibiotics for combating this disease, with a focus on identifying effective bioactive compounds.

METHODOLOGY

Selection of the targeted drug against *Yersinia ruckeri*

Yersinia ruckeri is an organism that possesses the pathogenic protein. DrugBank Online was the database utilised for identifying the pathogen's preferred therapeutic targets.[15–17] From this database, we downloaded the protein sequences in FASTA file format.

Homology modeling of the selected Drug Target

Obtaining the Homology Model of the selected or chosen Drug Target is essential. This is so because the data obtained from BLAST (uniprot.org) is raw. For the Homology Modelling, we use another web server known as Swiss Model (https://swissmodel.expasy.org/), which helps in modelling the desired protein and used to obtain the homologous structure of the protein. Among all the amino acids found in *Yersinia ruckeri*, three proteins, namely Tyrosine protein phosphatase YopH (PDB ID-1LYV), Putative stringent starvation protein (PDB ID- 1YY7) and Serine acetyltransferase were finalized as the possible drug targets based on interpretations from Ramachandra plots.

Retrieval of various phytochemical from IMPPAT Database

Phytochemicals from *Catharanthus rosueus* were selected as ligands. The chemical structures of different phytochemicals were obtained with the help of another database known as Imppat (https://cb.imsc.res.in/imppat/). Later, the 3D structure/ 2D structures of the phytochemicals were downloaded in the .sdf format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/ compound/5282184)[18]

Docking Analysis

The software that was used for docking the selected proteins with the various phytochemicals was PyRx. The proteins are converted to a macromolecule form and all the ligands are minimized using the open babel software available in PyRx. The AutoDocking was carried out with the help of an in built AutoDock Vina software.[19] PyMol software was used for visualizing the final docked Protein-Ligand complex.[20] This was enhanced by highlighting the polar, non-polar and the ligand molecules with prominent colors. The image obtained was saved in .png format and can be found attached below in the results section.

ADMET analysis of the Phytochemicals

The ADMET analysis, or the absorption, digestion, metabolism, excretion, and toxicity analysis[21], was carried out for the top 50 phytochemicals, portraying the highest binding energy. This was primarily done to see the efficiency of the phytochemicals, to check the drug likeliness and toxicity and to determine the pharmacodynamics and the pharmacokinetics against the specimen under the study. This analysis used the SwissADME server (http://www.swissadme.ch/) to confirm whether the phytochemicals obeyed the Lipinski's rule of five. Based on the several permeability factors obtained from admetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict/), the top six phytochemicals from one single species of plant were further analysed[21]. At the end, one single phytochemical that

passed all the permeability factors and that portrayed a potential greater than that of the standard was finalized as the potent or the most desirable phytochemical.

RESULTS

Selected Drug Targets

Three proteins, Tyrosine protein phosphatase, Putative stringent starvation and Serine acetyltransferase, were selected. The PDB models[22] were obtained for the two proteins: Tyrosine protein phosphatase and Putative stringent starvation. Swiss Model Database was used to obtain the homology modelling[23–26] of the Serine acetyltransferase protein. After the modelling, the Ramachandran Plot (shown in Figure 1) was obtained in which the favored regions for the ligands to bind to the targets were represented as the dotted region. The dots outside the model represented the dissimilar sequences.

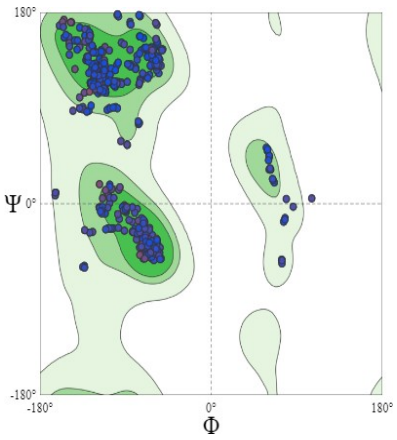


Figure 1 Ramachandran Plots for selected Drug Targets; Serine acetyltransferase (97.39%).

Database of the Phytochemicals that act as the Ligands

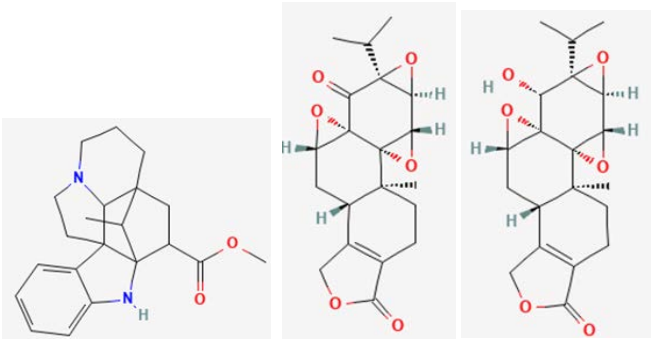
A single species of Indian Ethnobotanical plant, *Catharanthus roseus* was selected from which 335 phytochemicals were collected.

Docking of the Protein and Ligand

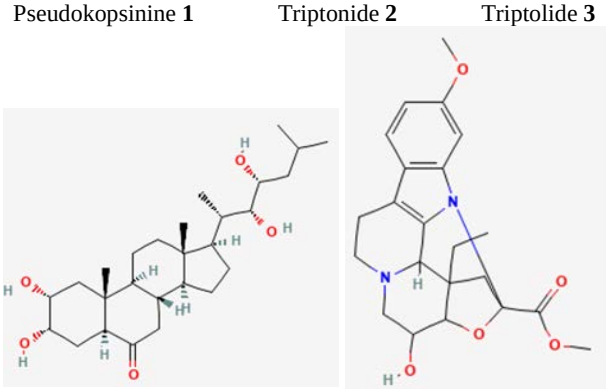
Proteins were docked using PyRx software and the following results were obtained. The results for the top six phytochemicals with the protein are shown in Table 1, 2 and 3.

Table 1 Docking results for the top 6 phytochemicals with Tyrosine protein phosphatase YopH (PDB ID- 1LYV)

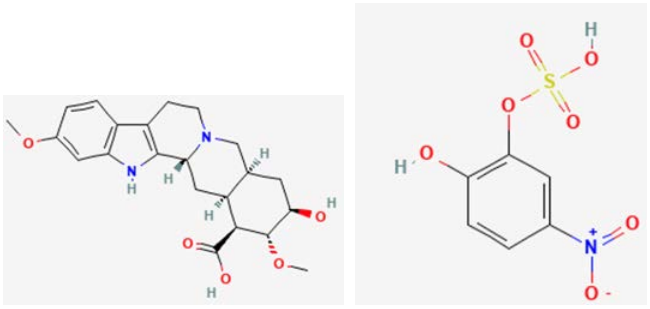
S.No.	Phytochemical	Binding energy (kcal/mol)	Molecular weight (g/mol)
1	Pseudokopsinine	-9.4	338.4
2	Triptonide	-8.7	358.4
3	Triptolide	-8.6	360.4
4	28-Norcastasterone	-8.1	450.7
5	Vincarodine	-8.1	398.5
6	Reserpic acid	-8.1	400.5
7	**4-Nitrocatechol sulfate (standard)**	-6.6	235.17



Pseudokopsinine **1** Triptonide **2** Triptolide **3**



28-Norcastasterone **4** Vincarodine **5**

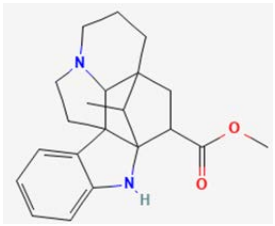


Reserpic Acid **6** 4-nitrocatechol **7**

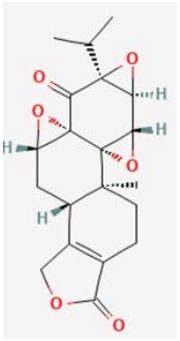
Figure 2 Ligands used Tyrosine protein phosphatase YopH

Table 2 Docking results for the top 6 phytochemicals with Putative stringent starvation protein (PDB ID-1YY7).

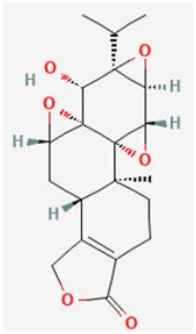
S.No.	Phytochemical	Binding energy (kcal/mol)	Molecular weight (g/mol)
1	Pseudokopsinine	-10.6	338.4
2	Triptonide	-8.8	358.4
3	Triptolide	-8.7	360.4
4	24-Epibrassinolide	-8.6	480.68
5	Pericyclivine	-8.6	322.40
6	Akuammigine	-8.6	352.43
7	**Citric acid (Standard)**	-5.4	192.12



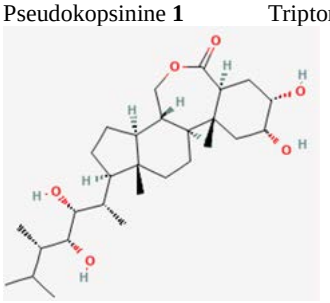
Pseudokopsinine **1**



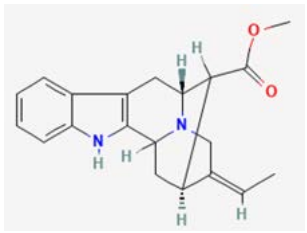
Triptonide **2**



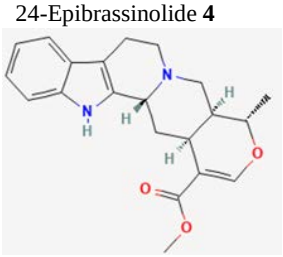
Triptolide **3**



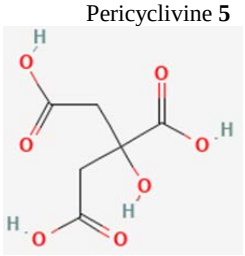
24-Epibrassinolide **4**



Pericyclivine **5**



Akuammigine **6**

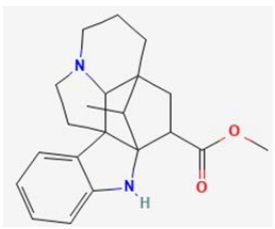


Citric Acid (Standard) **7**

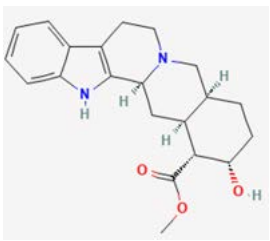
Figure 3 Ligands used for Putative stringent starvation protein

Table 3 Docking results for the top 6 phytochemicals with Serine acetyltransferase

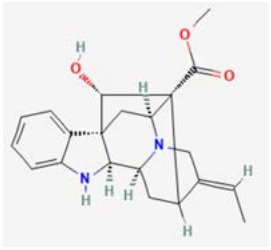
S.No.	Phytochemical	Binding energy (kcal/mol)	Molecular weight (g/mol)
1	Pseudokopsinine	-11	338.4
2	allo-Yohimbine	-9.4	354.4
3	Quebrachidine	-9.1	352.4
4	Vindolininol	-8.9	308.4
5	Serpentine (alkaloid)	-8.9	348.4
6	(+)-Condylocarpine	-8.8	322.4
7	**Triglyme (standard)**	-3.8	178.23



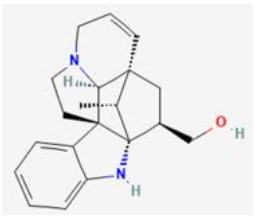
Pseudokopsinine **1**



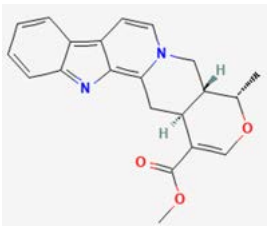
allo-Yohimbine **2**



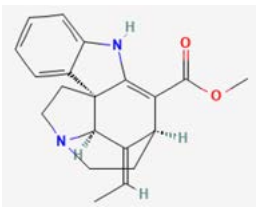
Quebrachidine 3



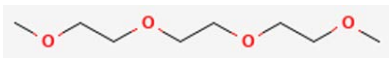
Vindolininol 4



Serpentine 5



(+) – Condylocarpine 6



Triglime 7 (standard)

Figure 4 Ligands used for Serine acetyltransferase

Physiochemical analysis of the top six results

Table 4 Comparative analysis of the Drug Likeness properties of top 6 Phytochemicals of *Catharanthus roseus* against Tyrosine protein phosphatase YopH obeying Lipinski's rule of 5

Drug Likeness property	Molecular weight (g/mol)	H-bond acceptor	H-bond donor	Molar refractivity	Total polar surface area (TPSA) A^2	Lipophilicity (Log Po/w)	Water solubility (Log S)
Standards	≤500	≤10	≤5	40-130	20-130	≤4.15	<-10
Pseudokopsinine	338.44	2	1	103.33	41.57	2.78	-3.89
Triptonide	358.39	6	0	87.58	80.96	1.94	-2.41
Triptolide	360.40	6	1	88.54	84.12	1.70	-2.15
28-Norcastasterone	450.65	5	4	127.77	97.99	3.53	-4.99
Vincarodine	398.45	6	1	109.20	73.16	1.93	-3.46
Reserpic acid	400.47	6	3	112.09	95.02	1.19	-1.93

Table 5 Comparative analysis of the Drug Likeness properties of top 6 Phytochemicals of *Catharanthus roseus* against Putative stringent starvation protein obeying Lipinski's rule of 5

Drug Likeness property	Molecular weight (g/mol)	H-bond acceptor	H-bond donor	Molar refractivity	Total polar surface area (TPSA) A^2	Lipophilicity (Log Po/w)	Water solubility (Log S)
Standards	≤500	≤10	≤5	40-130	20-130	≤4.15	<-10
Pseudokopsinine	338.44	2	1	103.33	41.57	2.78	-3.89
allo-Yohimbine	354.44	4	2	104.02	65.56	2.46	-4.01
Quebrachidine	352.43	4	2	103.98	61.80	1.92	-3.29
Vindolininol	308.42	2	2	97.92	35.50	2.40	-3.53
Serpentine	348.40	4	0	99.59	53.35	2.87	-3.86
(+)-Condylocarpine	322.40	3	1	99.91	41.57	2.59	-3.37

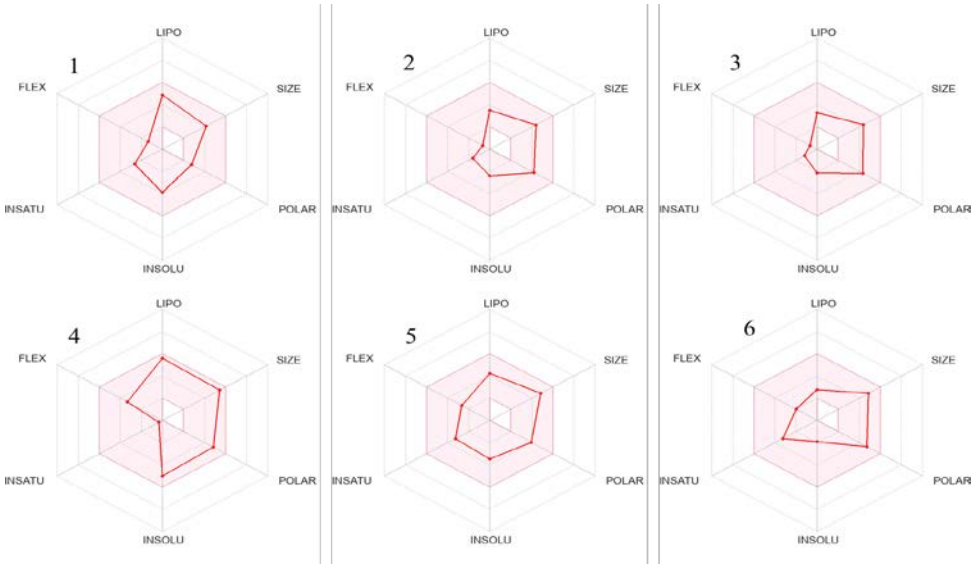


Figure 5 Oral bioavailability of the top 6 phytochemicals for drug usage against Tyrosine protein phosphatase YopH. Coloured zone represents the suitable physiochemical space for oral bioavailability

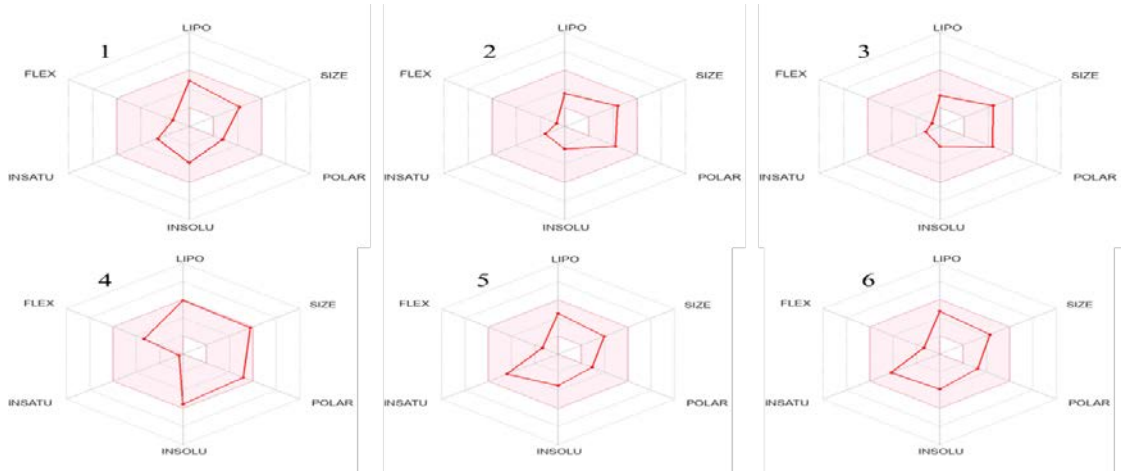


Figure 6 Oral bioavailability of the top 6 phytochemicals for drug usage against Putative stringent starvation protein: Coloured zone represents the suitable physiochemical space for oral bioavailability

Figure 7 Oral bioavailability of the top 6 phytochemicals for drug usage against Serine acetyltransferase: Coloured zone represents the suitable physiochemical space for oral bioavailability

Table 6 Comparative analysis of the Drug Likeness properties of top 6 Phytochemicals of *Catharanthus roseus* against Serine acetyltransferase obeying Lipinski's rule of 5

Drug Likeness property	Molecular weight (g/mol)	H-bond acceptor	H-bond donor	Molar refractivity	Total polar surface area (TPSA) A^2	Lipophilicity (Log Po/w)	Water solubility (Log S)
Standards	≤500	≤10	≤5	40-130	20-130	≤4.15	<-10
Pseudokopsinine	338.44	3	1	103.33	41.57	2.78	-3.89
Triptonide	358.39	6	0	87.58	80.96	1.94	-2.41
Triptolide	360.40	6	1	88.54	84.12	1.70	-2.15
24-Epibrassinolide	480.68	6	4	133.67	107.22	3.68	-5.54
Pericyclivine	322.40	3	1	97.58	45.33	2.76	-3.48
Akuammigine	352.43	4	1	103.47	54.56	2.67	-3.88

ADME/T analysis

Table 7 Comparative ADME/T analysis of the top 6 Phytochemicals of *Catharanthus roseus* against Tyrosine protein phosphatase YopH obeying Lipinski's rule of 5

Properties	Pseudokopsinine	Triptonide	Triptolide	28-Norcastasterone	Vincarodine	Reserpic acid
Gastrointestinal (GI)absorption	High	High	High	High	High	High
Blood-Brain Barrier (BBB)	Yes	No	No	No	No	No
Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+	Caco2+
P- glycol protein substrate	No	No	Yes	Yes	No	No
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	No	No	No	Yes	Yes
CYP3A4 inhibitor	No	No	No	No	No	No
Log Kp (skin permeation) in cm/s	-6.22	-8.02	-8.34	-6.02	-7.40	-9.23
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert	1 alert: indol_3yl_alk
Brenk	0 alert	1 alert: Three-membered_heterocycle	1 alert: Three-membered_heterocycle	0 alert	0 alert	0 alert
AMES Toxicity	Non-AMES toxicity	Non-AMES toxicity	Non- AMES toxicity	Non- AMES toxicity	Non-AMES toxicity	Non-AMES toxicity
Carcinogens	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen
Biodegradation	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable
Acute Oral Toxicity	II	III	III	III	III	II
Fish Toxicity	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT

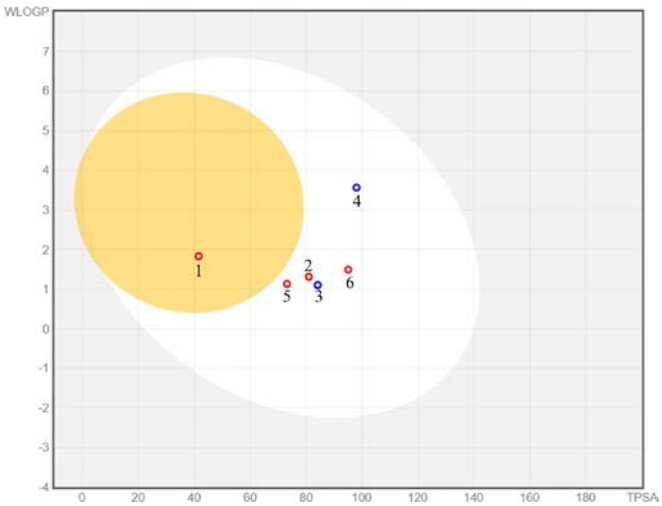


Figure 8 BOILED-Egg plot for phytochemicals against Tyrosine protein phosphatase YopH protein: Egg's white- Represents molecules predicted to absorb by the gastrointestinal tract passively. Points located in Egg's yolk- Represents molecules predicted to be passively permeate through the Blood brain barrier. Blue dots- Molecules predicted to be effluated from the central nervous system by the P-gylcoprotein. Red dots- Molecules predicted not to be effluated from the central nervous system by the P- gylcoprotein. 1) Pseudokopsinine; (2) Triptonide; (3) Triptolide; (4) 28-Norcastasterone; (5) Vincarodine; (6) Reserpic acid.

Table 8 Comparative ADME/T analysis of the top 6 Phytochemicals of *Catharanthus roseus* against Putative stringent starvation protein obeying Lipinski's rule of 5

Properties	Pseudokopsinine	Triptonide	Triptolide	24-Epibrassinolide	Pericyclivine	Akuammigine
Gastrointestinal (GI) absorption	High	High	High	High	High	High
Blood- Brain Barrier (BBB)	Yes	No	No	No	Yes	Yes
Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2-	Caco2+	Caco2+
P- glycoprotein substrate	No	No	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	No	No	No	Yes	Yes

CYP3A4 inhibitor	No	No	No	No	No	No
Log K_p (skin permeation) in cm/s	-6.22	-8.02	-8.34	-5.80	-6.58	-6.50
PAINS	0 alert	0 alert	0 alert	0 alert	1 alert: indol_3yl_alk	1 alert: indol_3yl_alk
Brenk	0 alert	1 alert: Three-membered_heterocycle	1 alert: Three-membered_heterocycle	0 alert	1 alert: isolated_alkene	0 alert
AMES Toxicity	Non-AMES toxicity	Non- AMES toxicity	Non-AMES toxicity	Non-AMES toxicity	AMES toxic	Non-AMES toxicity
Carcinogens	Non-carcinogen	Non- carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen
Biodegradation	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable
Acute Oral Toxicity	II	III	III	III	III	III
Fish Toxicity	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT

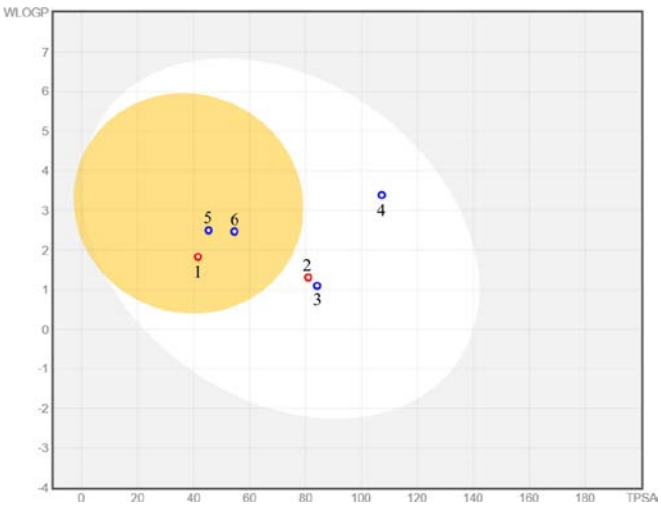


Figure 9 BOILED-Egg plot for phytochemicals against Putative stringent starvation protein: Egg's white- Represent molecules predicted to passively absorb by the gastrointestinal tract. Points located in Egg's yolk- Represents molecules predicted to be passively permeate through the Blood brain barrier. Blue dots- Molecules predicted to be effluated from the central nervous system by the P-gylcoprotein. Red dots- Molecules predicted not to be effluated from the central nervous system by the P- gylcoprotein. (1) Pseudokopsinine; (2) Triptonide; (3) Triptolide; (4) 24-Epibrassinolide; (5) Pericyclivine; (6) Akuammigine.

Table 9 Comparative ADME/T analysis of the top 6 Phytochemicals of *Catharanthus roseus* against Serine acetyltransferase obeying Lipinski's rule of 5

Properties	Pseudokopsinine	allo-Yohimbine	Quebrachidine	Vindoline inol	Serpentine (alkaloid	(+)-Condylocarpine
Gastrointestinal (GI) absorption	High	High	High	High	High	High
Blood-Brain Barrier (BBB)	Yes	Yes	No	Yes	Yes	Yes

Caco-2 Permeability	Caco2+	Caco2+	Caco2+	Caco2-	Caco2+	Caco2+
P- glycoprotein substrate	No	Yes	Yes	Yes	No	Yes
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	Yes	Yes
CYP2C9 inhibitor	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	Yes	Yes	Yes	Yes	Yes
CYP3A4 inhibitor	No	No	No	No	Yes	Yes
Log Kp (skin permeation) in cm/s	-6.22	-6.37	-7.07	-6.32	-6.59	-6.61
PAINS	0 alert	1 alert: indol_3yl_alk	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	0 alert	1 alert: isolated_alkene	1 alert: isolated_alkene	0 alert	1 alert: isolated_alkene
AMES Toxicity	Non-AMES toxicity	Non-AMES toxicity	Non-AMES toxicity	Non-AMES toxicity	AMES toxic	Non-AMES toxicity
Carcinogens	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen	Non-carcinogen
Biodegradation	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable	Not readily biodegradable
Acute Oral Toxicity	II	II	III	III	III	III
Fish Toxicity	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT	High FHMT

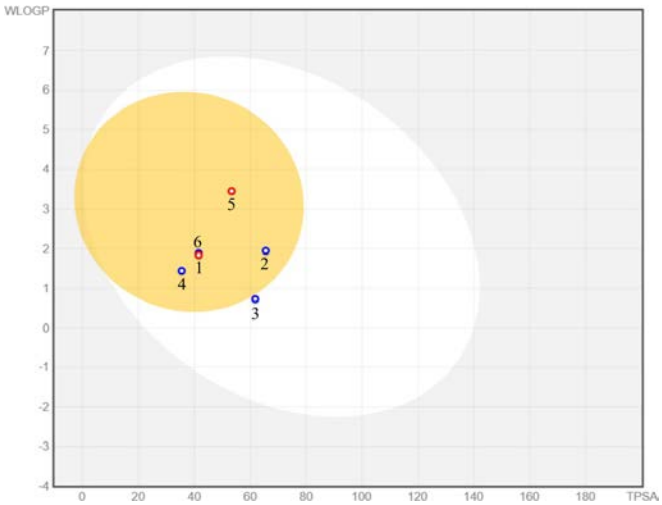


Figure 10 BOILED-Egg plot for phytochemicals against Serine acetyltransferase protein: Egg's white- Represent molecules predicted to absorb by the gastrointestinal tract passively. Points

located in Egg's yolk- Represents molecules predicted to be passively permeate through the Blood brain barrier. Blue dots- Molecules predicted to be effluated from the central nervous system by the P-gylcoprotein. Red dots- Molecules predicted not to be effluated from the central nervous system by the P-glycoprotein. (1) Pseudokopsinine; (2) allo-Yohimbine; (3) Quebrachidine; (4) Vindolininol; (5) Serpentine (alkaloid); (6) (+)-Condylocarpine.

PyMOL docking analysis

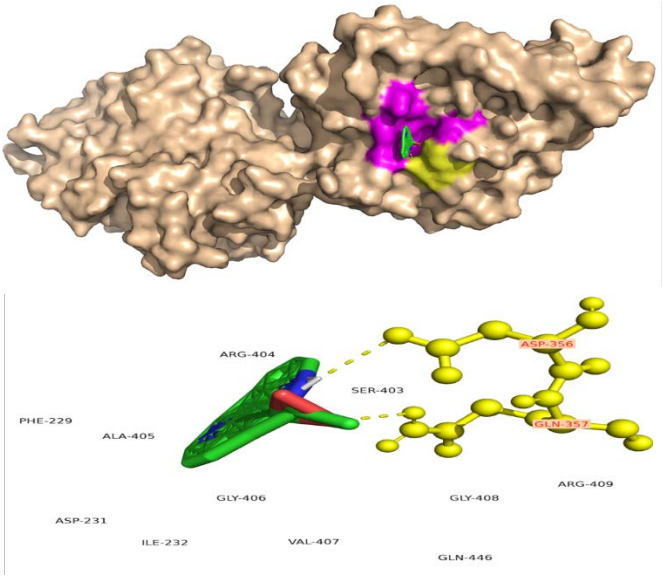


Figure 11 a) Docking interaction of Pseudokopsinine with Tyrosine protein phosphatase YopH

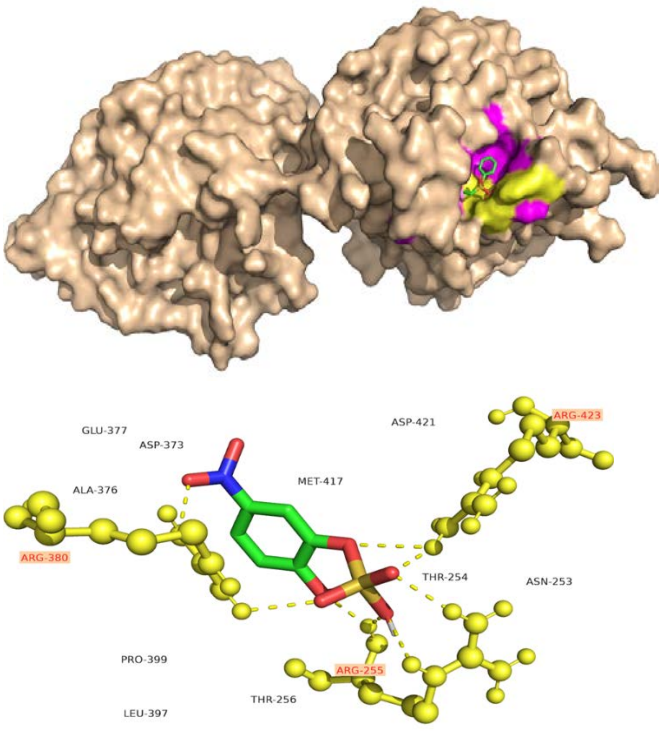


Figure 11b) Docking interaction of the 4-Nitrocatechol sulfate (Standard) with YopH. Color representation: Yellow: Polar bonds; Magenta: Non-polar bonds

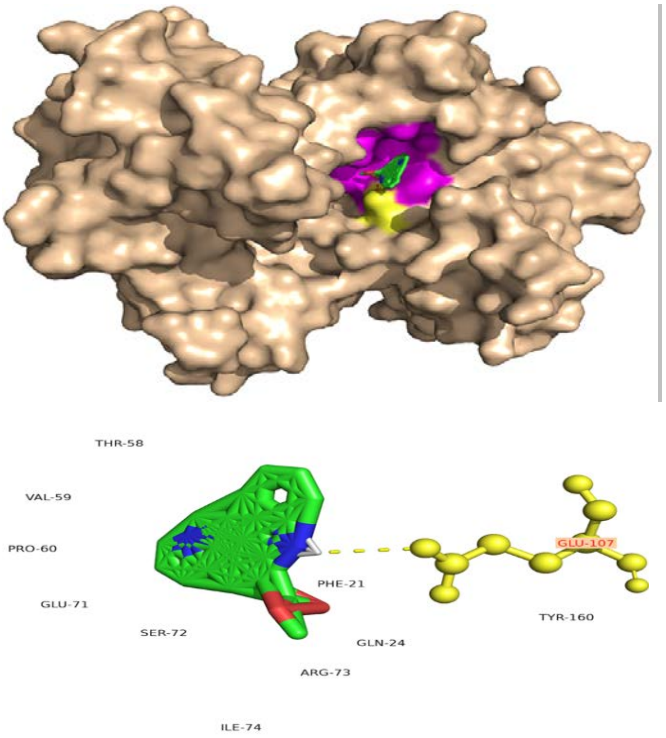


Figure 12 a) Docking interaction of the Pseudokopsinine with Putative stringent starvation protein

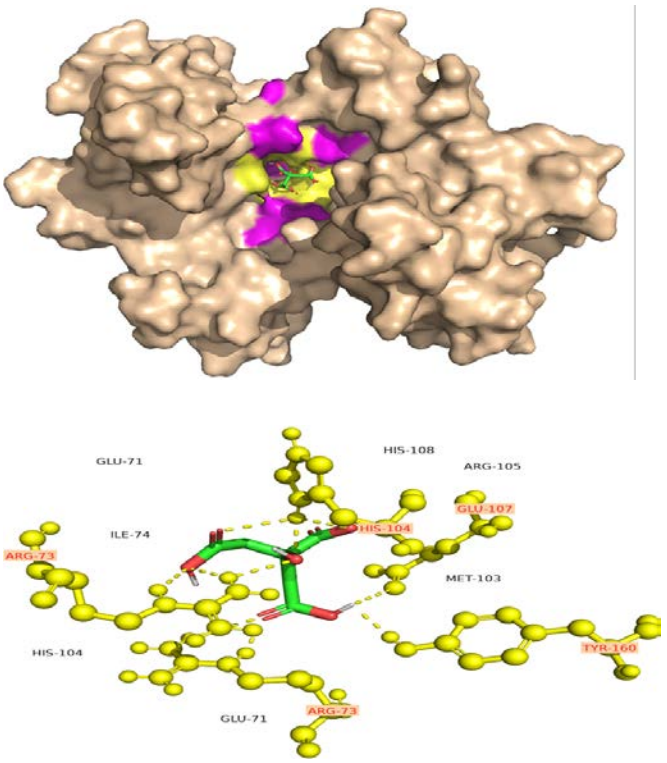


Figure 12 b) Docking interaction of the Citric acid (Standard) with Putative stringent starvation protein Color representation: Yellow: Polar bonds; Magenta: Non-polar bonds

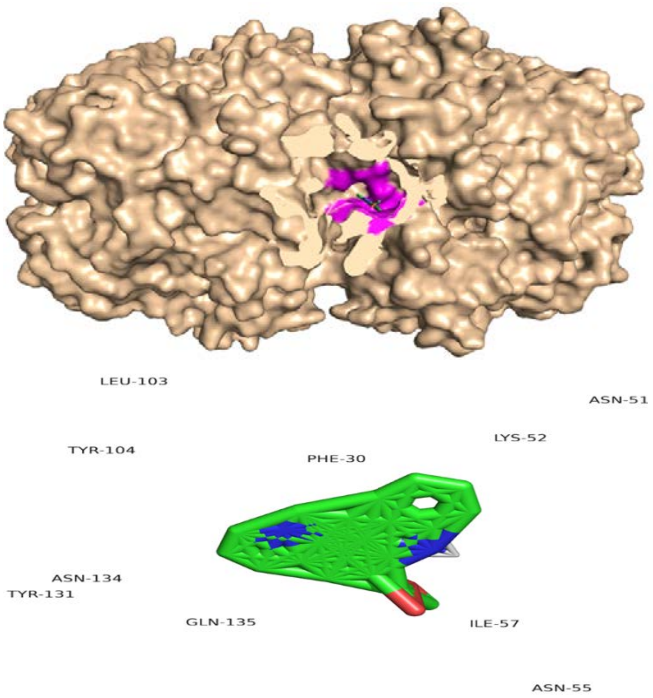


Figure 13 a) Docking interaction of the Pseudokopsinine with Serine acetyltransferase

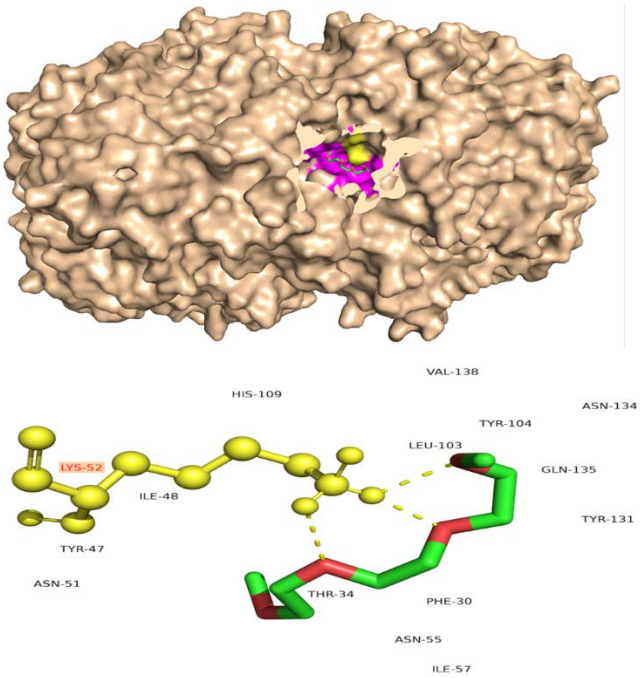


Figure 13 b) Docking interaction of the Triglyme (Standard) with Serine acetyltrasferase Color representation: Yellow: Polar bonds; Magenta: Non-polar bonds

DISCUSSION

In our research study we took three proteins namely Tyrosine protein phosphatase YopH (1LYV), Putative stringent starvation protein (1YY7) and Serine acetyltransferase (97.39%) in order to perform docking studies.

Tyrosine protein phosphatases (PTPs) (1LYV), (1YY7) play a crucial role in the regulation of numerous cellular functions, including as proliferation, differentiation, motility, cell-cell interactions, metabolism, gene transcription, and immunity.[27,28] In living beings, tyrosine phosphorylation is a dynamic and reversible process. Protein tyrosine phosphatases (PTPs) are responsible for dephosphorylation. Protein tyrosine kinases catalyze protein tyrosine phosphorylation. Hundreds of protein kinases, protein phosphatases, and their substrates make up its complicated signal-transducing network.[29] Several diseases, including cancer and diabetes, have their roots in the malfunction or improper operation of this network. Tyrosine protein phosphatase YopH was docked first, and the first fifty phytochemicals were analysed using the Drug likeliness[30] report (Table 4). According to this analysis, six phytochemicals were shortlisted and were screened using the ADME/T report (Table 7). The results showed that the phytochemical named Pseudokopsinine could effectively pass through the Blood Brain Barrier (BBB). The blood is the carrier for a phytochemical to be used as a drug. This could be understood through the Boiled Egg Model (Figure 5) wherein the yellow region in the model depicts the Blood Brain Barrier, and the white region depicts the Gastrointestinal Tract absorption.[31] Pseudokopsinine is the only potent observed phytochemical for the Tyrosine protein phosphatase YopH; it is interpreted to be the most suitable phytochemical compared with the other phytochemicals that passed through the ADME/T analysis.[32,33] Other statements to support the following inference includes the fact that Pseudokopsinine has (i) high gastrointestinal tract absorption, (ii) it has a positive $Caco^{2+}$ permeability ($Caco^{2}$ mimics as an intestinal membrane)[34], (iii) it has zero alerts for both PAINS (Pan assay interference compounds) and Brenks. Brenks primarily deals with the toxicity, reactivity or instability of the phytochemicals and (iv) compared to the other five phytochemicals it showed inhibitory effect against only one cytochrome[35], namely CYP2D6. The data can also be validated with the available standard (Table 1) which is 4-Nitrocatechol sulfate in this case. The binding energy of 4- Nitrocatechol sulfate is -6.6 kcal/mol, whereas Pseudokopsinine is -9.4kcal/mol. Comparatively, Pseudokopsinine shows a better inhibitory effect than the commercially available standards.

Putative stringent starvation was docked later, and the first fifty phytochemicals were analysed using the Drug likeliness report (Table 5). According to this analysis, six phytochemicals were shortlisted and were screened using the ADME/T report (Table 8). The results showed that the phytochemical named Pseudokopsinine could effectively pass through the Blood Brain Barrier (BBB). The blood is the carrier for a phytochemical to be used as a drug. This can be adequately understood through the Boiled Egg Model (Figure 6) wherein the yellow region in the model depicts the Blood Brain Barrier, and the white region depicts the Gastrointestinal Tract absorption. Pseudokopsinine is the only potent observed phytochemical for the Putative stringent starvation; it is interpreted to be the most suitable phytochemical compared with the other phytochemicals that passed through the ADME/T analysis.[36–40] Other statements to support the following

inference includes the fact that Pseudokopsinine has (i) high gastrointestinal tract absorption, (ii) it has a positive $Caco^{2+}$ permeability, and (iii) it has zero alerts for both PAINS (Pan assay interference compounds) and Brenks and (iv) compared to the other five phytochemicals it showed inhibitory effect against only one cytochrome, namely CYP2D6. The data can also be validated with the available standard (Table 2) which is Citric acid in this case. The binding energy of Citric acid is -5.4 kcal/mol, whereas Pseudokopsinine is **-10.6 kcal/mol**. Comparatively, Pseudokopsinine shows a better inhibitory effect than the commercially available standards.

Serine acetyltransferase is present in *Yersinia* species, and the target of this protein is not known as of now. Serine Acetyltransferase was docked finally, and the first fifty phytochemicals were analysed using the Drug likeliness report (Table 6). According to this analysis, six phytochemicals were shortlisted and were screened using the ADME/T report (Table 9). The results showed that the phytochemical named Pseudokopsinine could effectively pass through the Blood Brain Barrier (BBB). The blood is the carrier for a phytochemical as a drug. This can be adequately understood through the Boiled Egg Model (Figure 7) wherein the yellow region in the model depicts the Blood Brain Barrier, and the white region depicts the Gastrointestinal Tract absorption. Pseudokopsinine is the only potent observed phytochemical for the Serine Acetyltransferase; it is interpreted to be the most suitable phytochemical compared with the other phytochemicals that passed through the ADME/T analysis. Other statements to support the following inference includes the fact that Pseudokopsinine has (i) high gastrointestinal tract absorption, (ii) it has a positive $Caco^{2+}$ permeability, and (iii) it has zero alerts for both PAINS (Pan assay interference compounds) and Brenks and (iv) compared to the other five phytochemicals it showed inhibitory effect against only one cytochrome, namely CYP2D6. The data can also be validated with the available standard (Table 3) which is Triglyme in this case. The binding energy of Triglyme is -3.8 kcal/mol, whereas Pseudokopsinine is **-11 kcal/mol**. Comparatively, Pseudokopsinine shows a better inhibitory effect than the commercially available standards.

Corelating all the in-silico studies, we can infer that Pseudokopsinine was the most suitable phytochemical that can be extracted out of *Catharanthus roseus* to help treat the infectious enteric red mouth disease that is caused by *Yersinia ruckeri.*

CONCLUSION

Yersinia ruckeri, a Gram-negative rod shaped bacterium responsible for enteric red mouth disease, which causes significant economic losses, particularly in salmonid aquaculture. In order to mitigate this problem, we performed homology modelling, ADMET analysis, BOILED-Egg plot for phytochemicals and molecular docking analysis by using PyRx software with the three proteins namely Tyrosine protein phosphatase YopH, Putative stringent starvation and Serine acetyltransferase and docked with the phytochemicals retrieved from *Catharanthus roseus.* From our findings, we conclude that pseudokopsinine derived from *Catharanthus roseus* has a substantial inhibitory potential than the other commercially available standards and could be used to prevent enteric red mouth illness in Salmonid fishes.

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Author Contribution

Prof. Dr. Suneetha Vuppu prepared the manuscript backbone, obtained the homology of selected drug target, indexing different phytochemical database, molecular docking and ADMET analysis of phytochemicals along with Anushka Das, B Stany, Shatakshi Mishra, Kritika Srivastava. Prof. Dr. Suneetha Vuppu and Sathvika Kamaraj prepared the biorender diagrams and graphical abstract. The manuscript was read and analytically revised by all of the authors for important intellectual content.

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Conflict of Interest

The authors declare no conflict of interest

Competing Interests

The authors declare no competing interests

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