Pharmacokinetic and Molecular docking studies of *Centella asiatica* phytocompounds to explore potential anti-tuberculosis activity

[1]Narayan Sarkar[*] [2]Dhruba Som, [3]Saurov Mahanta, [4]Bhaben Tanti, [5]Mami Das

[1] Gauhati university, *([1]sarkarnarayn8@gmail.com)*
[2]IISER, Berhampur, *(dhruba.sm05@gmail.com)*
[3]NIELIT , GUWAHATI, *(saurov.mahanta@gmail.com)*
[4]Dept of Botany, Gauhati University, *(btanti@gauhati.ac.in)*
[5]Gauhati university

Abstract: ***Tuberculosis is caused by the bacterium Mycobacterium tuberculosis. The first line drugs available for treatment of TB are isoniazid, rifamsin etc. Second line drugs are Paraminosalicylate, kanamycin etc. Numerous reports have demonstrated the cause and emergence of multi-drug resistance of Mycobacterium tuberculosis.It is necessary to discover new drugs to control these new strains of M.tuberculosis. Computer aided drug discovery(CADD) is a new approach to discover new drugs from plants or other sources by the help of digital technologies. First step of this process is to select a suitable drug target. Next screening of the ligand against these targets to determine the binding affinities of these ligands and receptors. In this study, the two enzymes of Mycobacterial shikimate pathway shikimate dehydrogenase and chorismate synthase were selected as drug targets. The 6 phytocompounds from traditionaly used medicinal plant Centella asiatica was screnned against these targets. Molecular docking study was done to check the receptor(protein)-ligand (phytocompounds) binding. The result of molecular docking study shows the diffrent binding affinities of these phytocompounds with these two enzymes. So these phytocompounds may inhibit the activity of these two enzymes and may block the shikimate pathway of M.tuberculosis. Later , to establish as drug molecules pharmacokinetic analysis of these phytocompounds was done. All these phytocompounds have drug like properties , they obey Lipinski's rule of 5 and have less toxicity.***

Keywords:Centella asiatica, molecular docking, shikimate pathway, pharmacokinetics

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1. INTRODUCTION

Tuberculosis (TB) is a contagious infectious disease caused in humans. The main causative agent of human tuberculosis (TB) is *Mycobacterium tuberculosis*. Tuberculosis generally affectsthe lungs, but can also affect other parts of the body. Most infections show no symptoms, in which case it is known as latent tuberculosis About 10% of latent infections progress to active disease which, if left untreated, kills about half of those affected. *M.tuberculosis* belongs to theActinomycetes is an obligate aerobe (Gram positive), small, rod-like, slender, straight or slightlycurved bacillus, non-motile, non-encapsulated, pleomorphic which do not form spores. Over onethird of the global human population is infected with *M. tuberculosis*, while 90% of tuberculosisinfections are latent, with no clinical symptoms, they remain a concern due to their potential for reactivation (Koch et al., 2018).

Multidrug-resistant (MDR) and extensively drug-resistant tuberculosis (XDR-TB) represent a challenge for diagnosis, treatment, prevention and rehabilitation of TB. The recently report from the World Health Organization (WHO) indicated that, worldwide, approximately 400,000 people developed MDR-TB, and even 8.5% of MDR-TB patients were infected with XDR-TB. The XDR-TB Shows resistance to isoniazid, rifampicin, any fluoroquinolone, and aminoglycoside. Drug resistance may worsen further with the emergence of resistance to at least one of the injectable agents (amikacin, kanamycin, capreomycin) or to fluoroquinolones (deemed pre-extensively Drug-resistant (pre-XDR)-TB by some authors or both (extensively drug-resistant(XDR)-TB) (Nunes et al., 2020).

Resistance and persistence are the major issues in TB control. The emergence of multidrug- resistant, extensively drug-resistant, and totally drug-resistant TB makes the disease exceptionally difficult to treat. Thus, there is an urgent need to develop new therapeutic drugs to target TB and the other diseases described above. There are several drugs available for thetreatment of TB. These drugs can be classified into two categories; first

line drugs such as,isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (EMB) etc., and second linedrugs like paraamino salicylate (PAS), kanamycin, cycloserine (CS), ethionamide (ETA) amikacin, capreomycin, thiacetazone, fluoroquinolones, etc. Currently TB therapy is known asDOTS (directly observed treatment, short-course) consists of an initial phase of treatment with 4drugs, INH, RIF, PZA and EMB, for 2 months daily, followed by treatment with INH and RIF foranother 4 months, three times a week. Treatment of drug-susceptible MTB is difficult already,requiring 6 to 9 months of combination therapy in idealcircumstances. TB is now common in many parts of the world and is very difficult to treat. An increase in the number of TB patients is attributable to the insufficient supply or low quality of anti‑TB drugs. In the treatment of tuberculosis one of the major hurdles has become the emergence of multi-drug resistance *M. tuberculosis*. In such circumstances, the second line drugs are prescribed in combination with DOTS which is very expensive, has to be administered for a longer duration and has significant side effects. Traditionally used drugs have earned a little success due to the time and cost involved in development of anti-tuberculosis drug. Numerous reports have demonstrated the cause and emergence of multidrug-resistance of *M. tuberculosis*. To improve the treatment of these strains there is a rising need to develop anti-TB effective drugs (Winder, 1970).

The appearance of an incurable from of TB is a frightening prospect that has potentially disastrous consequences for humanity. It is particularly worrying because only one new TB drug (bedaquiline) has been approved by the FDA since the 1960s. Various pharmaceutical companies encounter hurdles for the discovery of drugs for TB treatment. Clearly, the development of new classes of anti-TB drugs is of global importance. New targets are a priority, since attacking the bacterium using multiple strategies provides the best means to prevent resistance. In the treatment of tuberculosis one of the major hurdles has become the emergence of drug resistance *M.tuberculosis*. For these reasons, a fundamental goal of the TB field is to develop a regimen of new drugs with novel targets and mechanisms of action (MOA) capable of treating both drugsensitive and drug resistant TB in weeks rather than months or years. With the increasing number of cases of latent and drug resistant tuberculosis, there is an urgent need to develop new, potentmolecules capable of combating this deadly disease (Gandhi et al., 2010).

From ancient times natural products are being considered as potent sources of antimicrobials because of their amazing chemical diversity. Plants and microbes have the potentiality to fight against environmental infections using their chemical arsenal of secondary metabolites and therefore many types of different structures have been reported to display an antimicrobial function. The shikimate pathway is one of the most important pathway in Mycobacterium which involves in the biosynthesis of aromatic amino acids and folate. And in this study we tried to inhibit some important enzymes involve in this pathway by some plant derived chemicals which are previously known to be effective in pulmonary diseases by computer aided drug discovery methods.And by this we can starved bacteria from getting aromatic amino acids, folates and other crucial compounds which are important for their survival (Nunes et al., 2020).

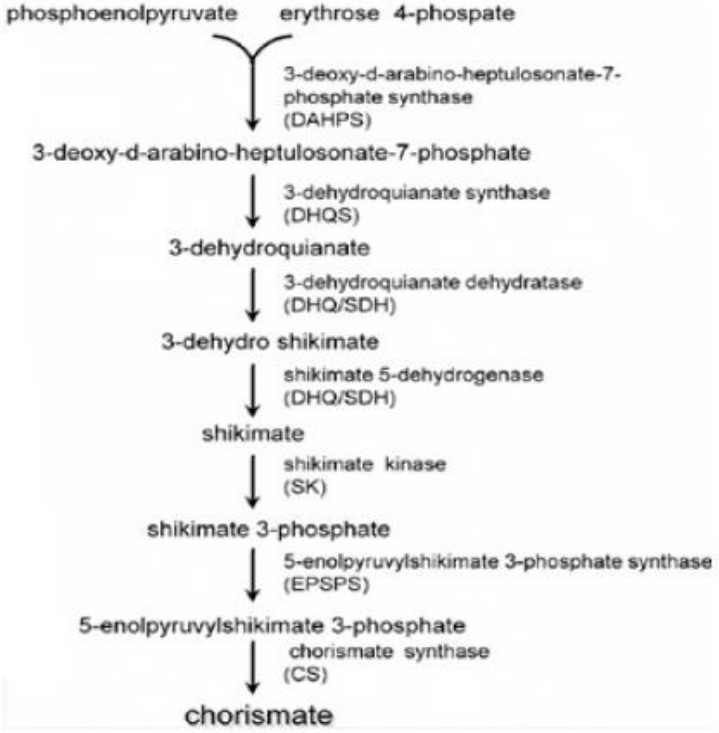


Fig 1: Shikimate Pathway of M. tuberculosis

Furthermore, *in silico* drug discovery is the process of discovering and designing lead compounds against the specific target proteins of the disease causing organism. It includes target identification, target validation, lead identification, lead optimization and introduction of the new drugs to the public. This is a novel approach to analyze the causes of the disease and to configure all the possible ways for the remedy. Therefore, the present investigation has been aimed to screen new ligands as drug candidates using different computationally based methods with the following objectives-

1. To screen novel lead compounds of *Centella asiatica* against the target proteins shikimate dehydrogenase(4P4G) and chorismate synthase(2O12) of shikimate pathway of *Mycobacterium tuberculosis* using DOCKING software package (Auto dock Vina).

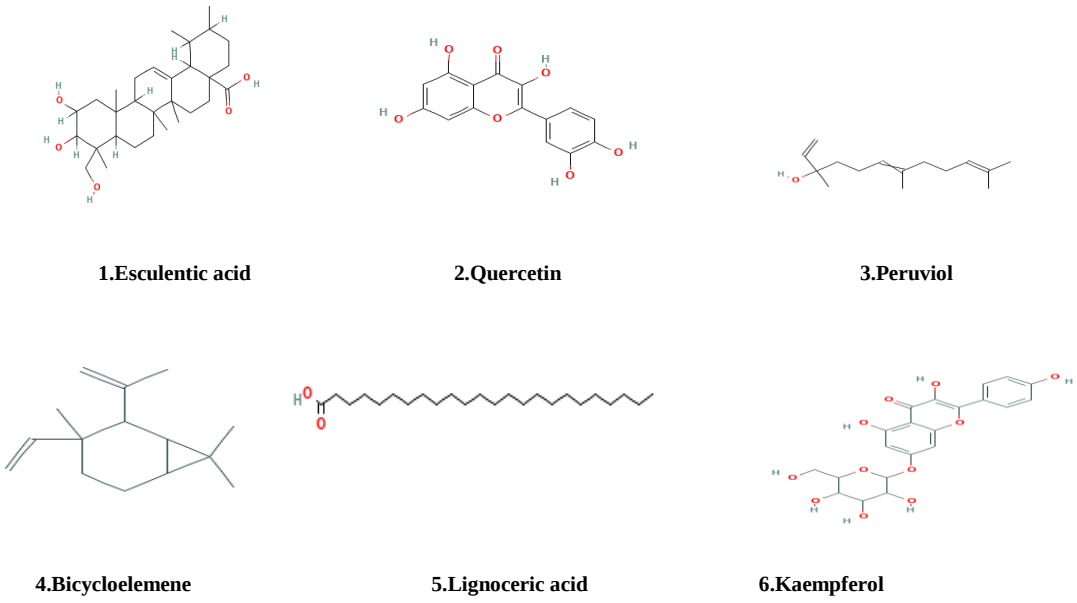
2. To study the drug like properties of the selected molecules including ADME-Tox studies to establish the selected molecules as potential lead molecules for discovery of novel antituberculosis drug(s).

2. MATERIALS AND METHODS

Preparation of target proteins:

The two target proteins shikimate dehydrogenase (PDB id: 4P4G) and chorismate synthase (PDB id: 2o12) was searched in RCSB PDB (rcsb.org). Their crystal structures were downloaded in PDB format. Shikimate dehydrogenase consists of two chains. It was found that all the chains had hundred percent similarities and hence chainA is used for the study. The binded ligands and water molecules from the target proteins were removed using UCSF chimera.The polar hydrogens were added. Energy minimization of the target proteins were done.

Preparation of the ligands: The GC-MS analysis was done to identify the phytocompounds from *Centella asiatica* plant. The result of GC-MS analysis was 6 phytocompounds from *Centella asiatica* plant. Using Accelrys Draw 4.2, the 2-D structures and chemical properties of the ligands used in this study were constructed. These 6 phytocompounds were used for molecular docking analysis and pharmacokinetic analysis. The torsions of the ligands were set by TORSDOF utility in autodock tools. Finally the ligands were prepared in PDBQT docking format.



1.Esculentic acid **2.Quercetin** **3.Peruviol**

4.Bicycloelemene **5.Lignoceric acid** **6.Kaempferol**

Fig 2: The 2D structures of the ligands

Molecular docking: The molecular docking analysis of all 6 phytocompounds of *Centella asiatica* was performed. These ligands were docked with selected targets (enzymes of shikimate pathway) shikimate

dehydrogenase (4P4G) and chorismate synthase (2012) using Autodock vina software (https://vinascrits.). The result shows different binding affinity of these ligands (phytocompounds) with the target proteins.

Visualization of the protein-ligand interaction: The receptor-ligand interactions were visualized using PYMoL . It was used to produce the images of the receptor-ligand complexes with polar (hydrogen bond) and non-polar interactions.

Analysis of pharmacokinetic properties of the ligands: To determine the pharmacokinetic (drug-like) properties of the ligands swiss ADME-Tox studies were done (http://www.swissadme.ch) by providing SMILES strings of the ligands to the server. Boiled egg analysis was done to check G1-absorption and Blood-Brain Barrier Permeability to predict bioactivity score, an online tool Molinspiration was used. Oral toxicity of ligands were evaluated by ProTox-II server.

3. RESULTS AND DISCUSSION:

Molecular Docking:

The six phytocompounds of Centella asiatica plant was selected and molecular docking was done against shikimate dehydrogenase (4P4G) and chorismate synthase (2o12) using Autodock vina software. These six compounds show different binding affinities with these two target proteins. The 3D interactions were observed. The compound Quercetin (compound 3) has highest binding affinity with both the target proteins as listed in table1.

Table 1 Ligand-receptor interaction of natural compounds which has highest binding affinity with M. tuberculosis 4P4G and 2o12 proteins.

Sl. No.	PDB ID	Binding affinity(Kcal/mol)					
		Esculentic acid	Quercetin	Bicycloelemene	Lignoceric acid	Peruviol	Kaempferol
1	4P4G	-8.4	-9.2	-8.1	-	-	-8.8
2	2o12	-	-7.5	-7.2	-7.2	-6.9	-

* Lig, ligand number; PDB ID, Protein Data Bank ID

Visualization of the protein ligand interactions:

The binding interactions between phytocompounds and target proteins were visualized by PyMoL. The result shows that seven amino acids Ile214, Asp105, Leu 240, His 17, Thr 65, Tyr215, Asp216 are involved in interaction of Quercetin with shikimate dehydrogenase (4P4G) . On the other hand 11 amino acids are involved in interaction of Quercetin with chorismate synthase (2o12) . Quercetin binds to active sites of these two enzymes and inhibits their activity.

Table 2 Ligand-receptor interaction with group involved in interaction of the receptor

Sl.No.	PDB ID	Ligands	Amino acids involved with interactive group
1	4P4G	**Quercetin**	**Ile 214** **Asp 105** **Leu 240** **His 17** **Thr 65** **Tyr 215** **Asp216**
2	2o12	**Quercetin**	**Ala349** **Gln 256** **Val 353** **Arg46** **Arg 40** **Ala346** **Ala 350** **Ala 138** **Ile 317** **Ala 257** **Arg 341**

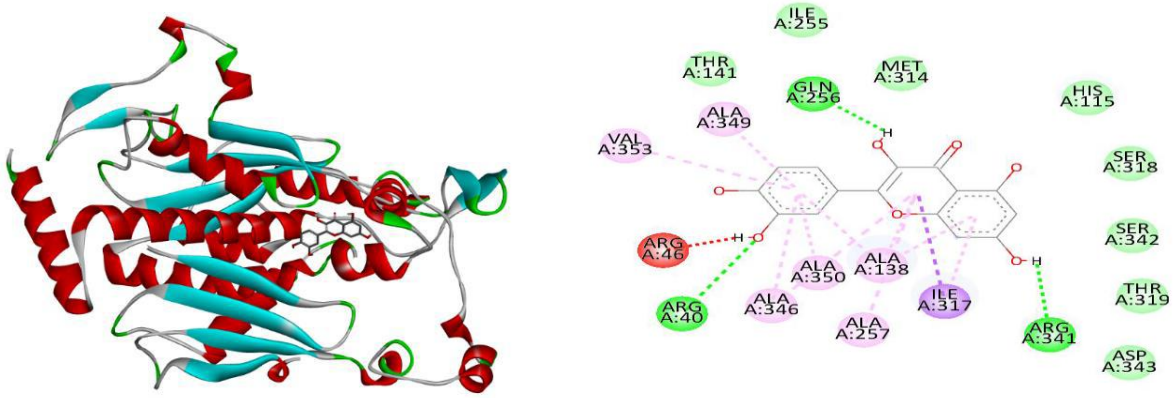


Fig 3. Interaction between receptor chorismate synthase (2o12) and Quercetin

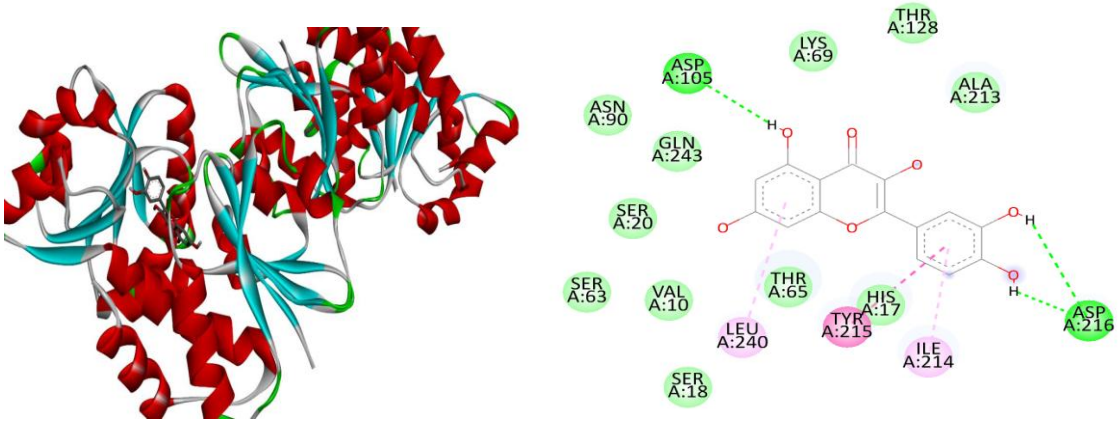


Fig 4. Interaction between receptor shikimate dehydrogenase (4P4G) and Quercetin

ADMET analysis of ligand(s)

The druglikeness of 6 phytocompounds of centella asiatica was listed in table 3. All the compounds have molecular weight (MW)<500 Da**.** However, except the compound kaempferol ,all the compounds obeyed the hydrogen bonding criteria of Lipinski's rule. Moreover, the lipophilicity(logP) and topological polar surface area(TPSA) values are crucial for forecasting oral liability of drug molecules. The ADMET analyses of phytocompounds were listed shown in Table 4.

Table 3 Pharmacokinetics properties of the natural compounds according to the Lipinski's rule analysis.

Sl. No.	Compound name	M.W. (g/mol)	No. of H bond acceptors	No. of H bond donor	RO5
1	**Esculentic Acid**	488.70	5	4	yes
2	**Quercetin**	302.24	7	5	yes
3	**Bicycloelemene**	204.35	0	0	yes
4	**Lignoceric acid**	368.64	2	1	yes
5	**Peruviol**	222.37	1	1	Yes
6	**Kaempferol**	448.38	11	7	No

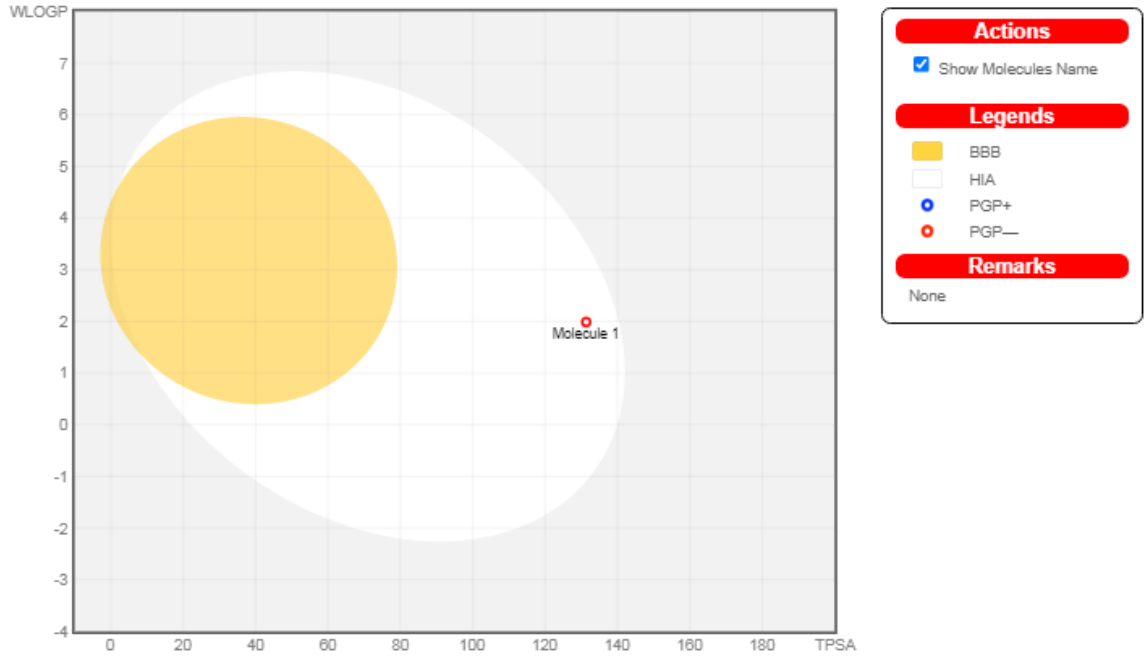
Table 4 ADMET properties of natural compounds for Centella asiatica plant

Sl. No.	Compound name	TPSA	natoms	nrotB	nVio
1	Esculentic Acid	97.99 Å²	35	2	0
2	Quercetin	131.36 Å²	22	1	0
3	Bicycloelemene	0.00 Å²	15	2	1
4	Lignoceric acid	37.30 Å²	26	22	1
5	Peruviol	20.23 Å²	16	7	0
6	Kaempferol	190.28 Å²	32	4	2

*TPSA, Topological Polar Surface Area; natoms, number of atoms; nrotB, number of rotatable bonds; nVio, number of Violations

BOILED EGG ANALYSIS

The boiled egg analysis evaluates the gastrointestinal absorption (HIA) and brain penetration(BBB) in function of the position of the molecules in the WLOGP versus –TPSA referential.The white region means the high probability of passive gastrointestinal absorption ang yellow portion means the high probability of brain penetration. The points are coloured in blue if predicted as actively effluxed by P-gp(PGP^+) and in red if predicted as non substrate of P-gp(PGP^-). The boiled egg analysis of Quercetin was done. This molecule is predicted as not absorbed and not brain penetrant(outside the egg), but PGP^-. The boiled egg analysis of this compound is shown in figure 5.



Bioactivity score prediction

The bioactivity or biological activity means the beneficial or adverse effects of a drug on living tissue.It suggests the uses of the phytocompounds in the medical applications. Molecules having bioactivity score more than 0.00 is most likely to exhibit considerable biological activity. If the values ranges from 0.50 to 0.00, are moderately active and if the score is less than 0.50, then it is inactive. Molinspiration tool was used to predict bioactivity score of Quercetin against human receptors such as GPCRs, ION CHANNEL, KINASE, NUCLEAR RECEPTORS, PROTEASES and ENZYMES, which is shown in the table5.

Sl. No.	Compound name	GPCR L	Ion CM	Kinase INH	Nuclear RL	Protease INH	Enzyme INH
1	Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28

Oral toxicity prediction

The prediction of compound toxicities is an important part of drug design development process. ProTox-II is a virtual lab for the prediction of toxicities of small molecules. Toxic doses are often given ad LD50 values in mg/kg body weight.The LD50 is the median lethal dose meaning the dose at which 50% of the test subjects die upon exposure to a compound.Toxicity classes are defined according to LD50
Class1: Fatal if swallowed(LD50<5)
Class2: Fatal if swallowed(5<LD50<50)
Class3:Toxic if swallowed(50<LD50<300)
Class4:Harmful if swallowed(300<LD50<2000)
Class5: May be harmful if swallowed(2000<LD50<5000)
Class6: Non toxic(LD50>5000)
The oral toxicity of the phytocompounds of this study was listed in Table 6. The compounds Esculentic acid and lignoceric acid belong to toxic class 4. Quercetin belongs to class3. Kaempferol , Peruviol and Bicycloelemene belong to toxic class 5.

Table 6 Oral toxicity prediction of natural compounds for *Centella asiatica* plant

Sl.No.	Compound name	LD50 (mg/kg)	Toxic. Class (1-6)	Avg. SM	pred. AC
1	Esculentic Acid	2000	Class4	89.49%	70.97%
2	Quercetin	159	Class3	100%	100%
3	Bicycloelemene	5000	class5	80.39%	70.97%
4	Lignoceric acid	900	class4	100%	100%
5	Peruviol	5000	class5	100%	100%
6	Kaempferol	3919	class5	82.46%	70.97%

*LD50, Lethal dose 50%; Toxic, Class- toxicity class; Avg. SM, Average similarity; Prediction accuracy

Molecular docking study of the phytocompounds of Centella asiatica plant against the two mycobacterial enzymes shows different binding affinities. The compound Quercetin has highest binding affinity with both the enzymes.The receptor ligand interactions are visualized by using PyMol software. Many amino acids are involved in these interactions. Later , Pharmacokinetic study was done and drug like properties of these phytocompounds was determined. The compounds have molecular weight less than 500g/mol, the number of rotatable bonds less than 5 and no. of atoms less than 10. So, these phytocompounds obey Lipinski's Rule of 5. To become a drug molecule , it must obey the Lipinski's rule. Boiled egg analysis of Quercetin was done . This compounds is not blood brain permeant and Gastro-intestinal absorption is also low. This compound is Bioactive against human receptors such as GPCRs, ION CHANNEL, KINASE, NUCLEAR RECEPTORS, PROTEASES and ENZYMES. LD50 of Quercetin is 159mg/kg , so it belongs to toxic class3 means that it may be toxic if swallowed.

4. CONCLUSION

This study deciphered that Quercetin exhibited the highest binding affinities with the two enzymes, shikimate dehydrogenase (4P4G) and chorismate synthase (2o12) of Mycobacterial Shikimate pathway, respectively. It was also evaluated that this compound could effectively bind to the active sites of these enzymes thereby inhibiting the functions of these enzymes.Thus, the Shikimate pathway would be interrupted and the bacterium would not produce aromatic amino acids for its survival.The enzymes of Shikimate pathway thus serve as novel drug target for the drug discovery process for treatment of TB. Besides, the above mentioned phytocompound Quercetin exhibited promising pharmacokinetic properties,obeyed Lipinski's rule of 5, with no violation and also conferred less toxicity (toxic class 3). Therefore, this molecule can be considered as potent drug molecules in TB treatment.

REFERENCES

[1] Alturki, M. S., Fuanta, N. R., Jarrard, M. A., Hobrath, J. V., Goodwin, D. C., Rants'o, T. A., andCalderón, A. I. (2018). A multifaceted approach to identify non-specific enzyme inhibition: Application to *Mycobacterium tuberculosis* shikimate kinase. *Bioorganic & Medicinal Chemistry Letters*, 28(4): 802-808

[2] Bylka, W., Znajdek-Awiżeń, P., Studzińska-Sroka, E., Dańczak-Pazdrowska, A., andBrzezińska, M. (2014). *Centella asiatica* in dermatology: an overview. *Phytotherapy Research*, 28(8): 1117-1124.

[3] Cazzaniga, G., Mori, M., Chiarelli, L. R., Gelain, A., Meneghetti, F., and Villa, S. (2021). Natural products against key Mycobacterium tuberculosis enzymatic targets,emerging opportunities for drug discovery. European Journal of Medicinal Chemistry, 113732.

[4] Comess, K. M., McLoughlin, S. M., Oyer, J. A., Richardson, P. L., Stö ckmann , H., Vasudevan,A., and Warder, S. E. (2018). Emerging approaches for the identification of proteintargets of small molecules-a practitioners' perspective. *Journal of Medicinal Chemistry*,61(19): 8504-8535.

[5] Gordon, S., Simithy, J., Goodwin, D. C., and Calderón, A. I. (2015). Selective *Mycobacterium tuberculosis* shikimate kinase inhibitors as potential antibacterials. *Perspectives in Medicinal Chemistry*, 7: PMC-S13212.

[6] Kingdon, A. D., andAlderwick, L. J. (2021). Structure-based in silico approaches for drug discovery against *Mycobacterium tuberculosis*. *Computational and Structural Biotechnology Journal*, 19: 3708.

[7] Kumar, V., Sharma, A., Machawal, L., Nagarajan, K., and Siddiqui, S. A. (2014). Effect of Centella asiatica against anti-tuberculosisdrugs-induced hepatotoxicity. *Involvement of Mitochondria and Oxidative Stress Journal ofPhytopharmacol*, 3: 310-15.

[8] Vianna, C. P., and de Azevedo, W. F. (2012). Identification of new potential *Mycobacterium tuberculosis* shikimate kinase inhibitors through molecular docking simulations. *Journal of Molecular Modeling*, 18(2): 755-764.

[9] Mudaliana, S. (2021). Antimicrobial activity of *Centella asiatica* and *Gigantochloa apus*. *Journal of Basic and Clinical Physiology and Pharmacology*, 32(4): 755-759.

[10] Nunes, J. E., Duque, M. A., de Freitas, T. F., Galina, L., Timmers, L. F., Bizarro, C. V., and Ducati,R. G. (2020). *Mycobacterium tuberculosis* shikimate pathway enzymes as targets for the rational design of anti-tuberculosis drugs. *Molecules*, 25(6): 1259.

[11] Mehra, R., Rajput, V. S., Gupta, M., Chib, R., Kumar, A., Wazir, P., ... andNargotra, A. (2016).Benzothiazole derivative as a novel *Mycobacterium tuberculosis* shikimate kinase inhibitor: Identification and elucidation of its allosteric mode of inhibition. *Journal of Chemical Information and Modeling*, 56(5): 930-940.

[12] Holdgate, G. A., Meek, T. D., and Grimley, R. L. (2018). Mechanistic enzymology in drugdiscovery: a fresh perspective. *Nature Reviews Drug Discovery*, 17(2): 115-132.