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In silico Prediction of Phytoconstituents from the Gliricidia Sepium against Citrobacter Rodentium

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KEYWORDS

CB-Dock2, *Citrobacter rodentium, Gliricidia sepium*, Molecular Docking, PDB ID: 8SSK.

ABSTRACT:

Introduction: *Gliricidia sepium* is rich in flavonoids, phenolic acids, and phytosterols, which contribute to its diverse therapeutic applications.

Objectives: This study examines the chemical constituents from *Gliricidia sepium* to find out how they could be used as potential candidate, particularly their effect on the *Citrobacter rodentium* (PDB ID: 8SSK) protein.

Methods: To study how well the chosen bioactive compounds work and how they interact at a molecular level, researchers used computer methods like molecular docking and toxicity predictions. Molecular docking was performed using CB-Dock2, and toxicity predictions were conducted using ProTox 3.0. Warfarin and bromadiolone, known anticoagulants, were used as standard drugs for comparison.

Results: The molecular docking analysis showed that bromadiolone was the most strongly attracted to the target with a score of -11.5, followed by stigmasterol at -10.1 and β-sitosterol at -9.8. The compounds interacted with key amino acids such as PHE230, GLY232, TRP250, and ASP251. The ADME analysis showed diverse solubility and permeability profiles, with Log P values ranging from 0.50 to 8.02. Toxicity predictions indicated that most compounds were in classes 3 to 6, with LD50 values from 159 mg/kg for quercetin (class 3) to 70,000 mg/kg for coumarin (class 6). Standard drugs warfarin and bromadiolone exhibited significantly lower LD50 values, indicating higher toxicity. Additionally, hepatotoxicity, nephrotoxicity, and cardiotoxicity risks were assessed for each compound.

Conclusions: This study found several useful compounds from *Gliricidia sepium* that have good binding abilities and beneficial properties for how they move through the body. The results suggest that flavonoids, sterols, and phenolic acids may serve as potential drug candidates. Further in vitro and in vivo studies are required to validate their therapeutic potential and safety for pharmaceutical applications..

1. Introduction

Natural bioactive compounds have become popular in pharmaceutical research due to their potential therapeutic applications. *Gliricidia sepium* is recognized for having a lot of flavonoids, phenolic acids, and phytosterols, which help give it various medicinal benefits. These bioactive molecules play crucial roles in various biological processes and are being explored for their potential in drug discovery [1, 2]. The plant has important natural compounds like quercetin, kaempferol, luteolin, apigenin, and gallic acid, which are linked to benefits like fighting off free radicals, reducing inflammation, and killing germs. Additionally, phytosterols like β-sitosterol, stigmasterol, and lupeol, along with coumarins and hydroquinone, contribute to its potential therapeutic applications [3, 4]. These compounds have shown promising interactions with biological targets, making them ideal candidates for computational studies [5, 6]. This study aims to investigate the bioactive compounds of *Gliricidia sepium* using computational approaches. Molecular docking will be done with CB-Dock2 to see how well these compounds can attach to the 8SSK protein, which we got from the Protein Data Bank (PDB). ProTox 3.0

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will also be used to predict how toxic these compounds might be, looking at risks to the liver, nervous system, and kidneys [7]. The pharmacokinetic properties, including ADME analysis, will help determine the drug-likeness of these compounds. To assess their therapeutic potential, warfarin and bromadiolone will be used as standard drugs for comparison [8, 9]. By leveraging computational tools, this study aims to identify bioactive compounds with strong binding affinities and favorable pharmacokinetic properties. The findings will teach us about the potential application of these compounds in pharmaceutical formulations and pave the way for further experimental validation [10, 11].

2. Methods

Molecular Docking:

Ligand Selection: For this in silico study, bioactive compounds from *Gliricidia sepium* were selected due to their documented pharmacological properties. These compounds include quercetin, kaempferol, luteolin, apigenin, gallic acid, β-sitosterol, stigmasterol, lupeol, and others. Warfarin and bromadiolone were used as standard drugs for comparison [12, 13].

Protein of Choice: The *Citrobacter rodentium* (PDB ID: 8SSK) protein was chosen as the target due to its potential role in biological regulatory mechanisms. Natural bioactive compounds from *Gliricidia sepium* were evaluated for their ability to interact with this protein. Molecular docking and toxicity prediction methods were used to examine how these natural compounds from *Gliricidia sepium* interact with the *Citrobacter rodentium* (PDB ID: 8SSK) protein. Computational tools such as CB-Dock2 for molecular docking and ProTox 3.0 for toxicity assessment were employed to identify promising lead compounds [14-16].

Molecular Docking: Molecular docking was performed using CB-Dock2 to predict the binding interactions between bioactive compounds from *Gliricidia sepium* and the 8SSK protein. The structure of the *Citrobacter rodentium* (PDB ID: 8SSK) protein was obtained from the Protein Data Bank (PDB) and prepared by taking out water molecules and other non-protein parts. Active binding sites were identified based on the protein's structure. The molecular structures of the selected phytoconstituents were generated using Chem Draw software and optimized for docking. A receptor grid was created for ligand docking, and docking scores were recorded [17-19].

ADME Properties: The Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the selected phytoconstituents were analyzed to determine their drug-likeness and pharmacokinetic behaviour. These parameters gave us information about the bioavailability and metabolic stability of the compounds, helping to predict their potential as therapeutic agents [20-22].

Prediction of LD50 and Toxicity Class: The LD50 values (lethal dose 50%) and toxicity class were predicted by using ProTox 3.0 software. Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS) (Table 1). LD50 values are given in [mg/kg]:

Table 1: Globally harmonized system of classification of labelling of chemicals (GHS)

Class I	Fatal if swallowed	(LD50≤ 5)
Class II	Fatal if swallowed	(5<LD50≤ 50)
Class III	Toxic if swallowed	(50< LD50≤ 300)
Class IV	Harmful if swallowed	(300<LD50≤ 2000)
Class V	May be harmful if swallowed	(2000<LD50≤5000)
Class VI	Non-toxic	(LD50>5000)

***In silico* Metabolic Study:** The study was carried out in ProTox 3.0 software. The ligand molecules are drawn in ChemDraw software [23]. The cytochrome P450 enzymes (CYP enzymes) are essential for the metabolism of various substances, including drugs and dietary components. Each CYP enzyme is responsible for processing different sets of compounds. CYP1A2, CYP2C9, CYP2D6, CYP3A4, and CYP2E1 have distinct roles in drug metabolism and detoxification

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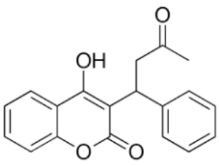
processes. Understanding which compounds interact with these enzymes can be important for predicting drug interactions, managing side effects, and personalizing medical treatments. This chart describes how different chemical constituents interact with various enzymes in the liver, called cytochrome P450 enzymes (CYP enzymes). These enzymes help break down drugs and other substances in the body. The chart shows which compounds are active and which are inactive [24].

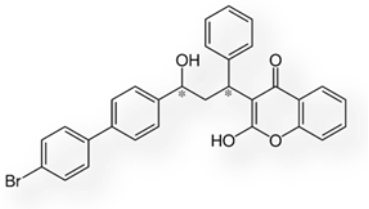
***In silico* Toxicity Prediction:** To evaluate the safety of the selected compounds, ProTox 3.0 software was used for toxicity assessment. The toxicological parameters, such as hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity, were analyzed. The toxicity classification of each compound was determined to assess their suitability for further pharmaceutical development [25].

3. Results

Following are the chemical constituents from Gliricidia sepium plant and molecules Warfarin and Bromadiolone taken as standard, represented in figure 1 and 2.

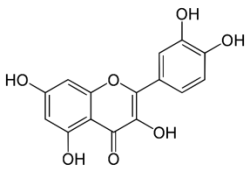


Warfarin

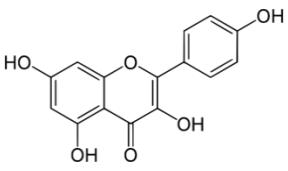


Bromadiolone

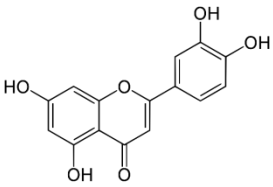
Figure 1: Structure of Warfarin and Bromadiolone taken as standard



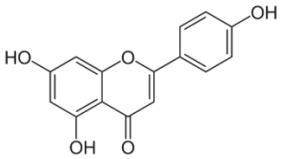
Quercetin



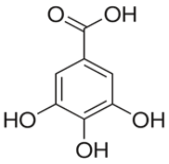
Kaempferol



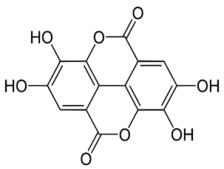
Luteolin



Apigenin

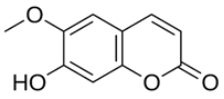


Gallic Acid

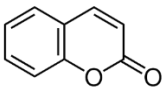


Ellagic Acid

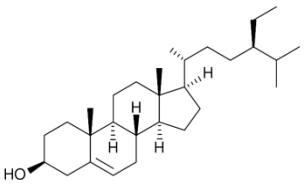
Hydroquinone



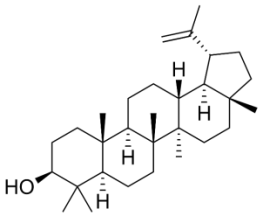
Scopoletin



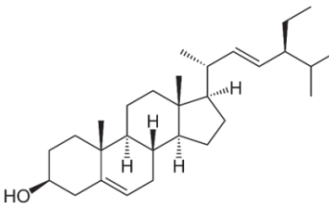
Coumarin



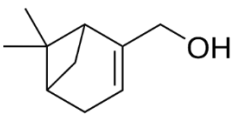
β-Sitosterol



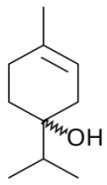
Lupeol



Stigmasterol



Myrtenol



Terpinen-4-ol

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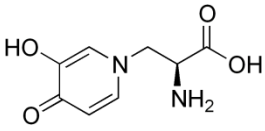

Mimosine

Figure 2: Chemical constituents present in *Gliricidia sepium* have been selected for molecular docking.

Molecular Docking:

Table 2: Molecular Docking Score and Surrounding Amino Acid Sequence in Chemical Constituents of *Gliricidia Sepium*

Sr. No	Name of Phyto-constituents	Molecular Docking Score	Surrounding Amino Acid Sequence
Standard			
1	Bromadiolone	-11.5	Chain A: PHE230 GLY232 PHE233 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295 ASN296
2	Warfarin	-8.8	Chain A: PHE230 GLY232 PHE233 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
Phytoconstituents			
1	Stigmasterol	-10.1	PHE230 GLY232 PHE233 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
2	β-Sitosterol	-9.8	PHE230 GLY232 PHE233 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
3	Kaempferol	-9.1	PHE230 VAL231 GLY232 PHE233 MET249 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 LYS287 PHE289 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
4	Lupeol	-9.0	PHE230 GLY232 PHE233 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295 ASN296
5	Apigenin	-9.0	PHE230 GLY232 PHE233 GLU234 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
6	Luteolin	-9.3	PHE230 GLY232 PHE233 GLU234 ASP237 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 ASP286 LYS287 GLU288 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
7	Quercetin	-8.1	PHE230 GLY232 PHE233 GLU234 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 LYS287 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
8	Ellagic Acid	-8.0	PHE230 TRP250 ASP251 GLN252 GLY253 SER254 LYS255 PHE289 LYS290 GLY291 THR292 TRP293 ASN294 GLY295
9	Gallic Acid	-6.9	SER130 GLY131 ALA132 ILE134 ALA136 LYS137 LEU138 LEU139 LYS156 GLY157 GLY158 GLY160 GLY161 ASN162

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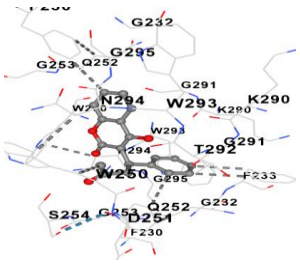
A detailed molecular docking study was done to check how well different plant compounds stick to the 8SSK protein. The docking scores and amino acid interactions were assessed to identify potential bioactive candidates. Among the compounds tested, stigmasterol (-10.1) and β-sitosterol (-9.8) showed strong binding to the 8SSK protein by connecting with important amino acids like PHE230, GLY232, PHE233, TRP250, and ASP251. Importantly, kaempferol (-9.1) connected with extra residues like VAL231, MET249, and PHE289, suggesting it might bind in a different way. Other flavonoids, like luteolin (-9.3) and apigenin (-9.0), showed similar binding strengths and interacted with important active site residues. Lupeol (-9.0) and quercetin (-8.1) also displayed significant interactions, though quercetin's affinity was slightly lower than other flavonoids. Among the compounds tested, ellagic acid (-8.0) and gallic acid (-6.9) had moderate scores for how well they fit, with gallic acid interacting with a different group of residues, including SER130, GLY131, and ALA132. In contrast, standard compounds like bromadiolone (-11.5) and warfarin (-8.8) showed strong interactions, providing a reference point for the plant compounds being studied (Table 2). The results indicate that the tested sterols and flavonoids might be able to block the *Citrobacter rodentium* (PDB ID: 8SSK) enzyme, which means more lab and live studies are needed to look into their possible medical uses.

Drug receptor interaction diagram:



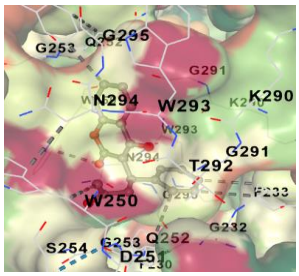
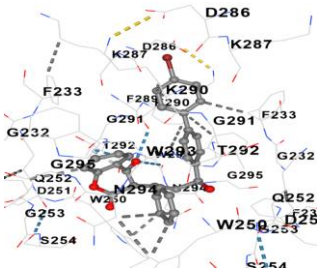


Figure 3a **Figure 3b**

Figure 3a: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with standard Warfarin in the hide receptor interaction was represented in 3D using CB Dock software.

Figure 3b: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with standard Warfarin in drug-receptor interaction was represented in 3D using CB Dock software.



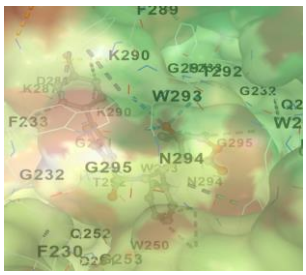
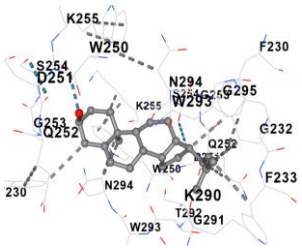


Figure 4a **Figure 4b**

Figure 4a: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with standard Bromadiolonein the hide receptor interaction was represented in 3D using CB Dock software.

Figure 4b: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with standard Bromadiolone in drug-receptor interaction was represented in 3D using CB Dock software.



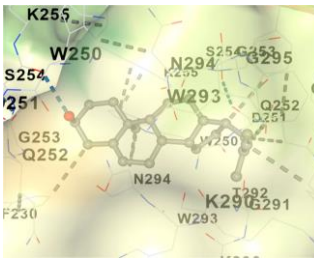


Figure 5a **Figure 5b**

Figure 5a: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with Stigmasterol in the hide receptor interaction was represented in 3D using CB Dock software.

Figure 5b: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with Stigmasterol in drug-receptor interaction was represented in 3D using CB Dock software.

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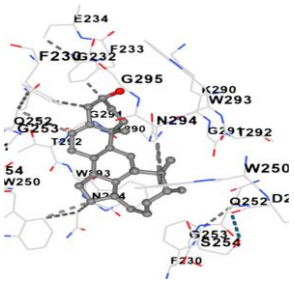

Figure 6a

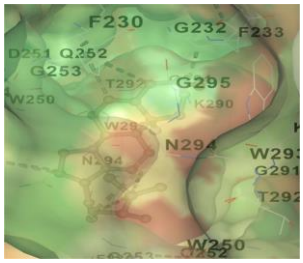

Figure 6b

Figure 6a: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with β-Sitosterolin the hide receptor interaction was represented in 3D using CB Dock software.

Figure 6b: The catalytic portion of *Citrobacter rodentium* (PDB ID: 8SSK) proteins with β-Sitosterolin drug-receptor interaction was represented in 3D using CB Dock software.

ADME PROPERTIES

Table 3: ADME Properties of *Gliricidia Sepium*

Compound	Mass (g/mol)	Log P	HBA	HBD	NRB	TPSA
Standard						
Warfarin	308.33	3.61	3	1	4	308.33
Bromadiolone	527.41	7.18	3	2	6	527.41
Phytoconstituent						
Quercetin	302.24	1.99	6	5	1	131.36
Kaempferol	371.52	6.00	2	0	8	12.47
Luteolin	170.12	0.50	5	4	1	97.99
Apigenin	302.19	1.31	6	4	0	141.34
Gallic Acid	110.11	1.10	2	2	0	40.46
Ellagic Acid	150.13	1.05	2	0	0	39.44
Hydroquinone	136.11	1.39	1	0	0	43.35
Scopoletin	372.63	7.00	1	1	5	20.23
Coumarin	426.72	8.02	1	1	0	20.23
β-Sitosterol	328.53	5.89	1	1	2	20.23
Lupeol	192.21	1.31	4	3	1	75.35
Stigmasterol	164.24	2.28	1	1	1	20.23
Myrtenol	140.22	1.97	1	1	0	43.95
Tea tree oil	328.53	5.89	1	1	2	20.23
Terpinen-4-ol	192.21	1.31	4	3	1	75.35

***HBD:** Hydrogen bond donar, **HBA:** Hydrogen bond donar, **TPSA:** Topological Polar Surface Area, **NRB:** Number of Rotatable bonds

Table 4: LD50 and Toxicity Class of *Gliricidia Sepium* Compounds

Sr. No.	Name of Phytoconstituent	Predicted LD50 (mg/kg)	Toxicity Class
Standard			
1	Warfarin	2	1
2	Bromadiolone	1	1
Phytoconstituent			
1	Quercetin	159	3
2	Kaempferol	1190	4
3	Luteolin	2000	4
4	Apigenin	2991	4
5	Gallic Acid	225	3
6	Ellagic Acid	1500	4
7	Hydroquinone	322	4
8	Scopoletin	890	4
9	Coumarin	70000	6
10	β-Sitosterol	750	4
11	Lupeol	3000	5
12	Stigmasterol	2340	5

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13	Myrtenol	14022	4
14	Tea tree oil	328.53	4
15	Terpinen-4-ol	372.63	4

Table 5: Metabolic Enzyme activity of phytoconstituents

Sr. No	Name of Phyto-constituent	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
Standard						
1	Warfarin	Inactive (0.72)	Inactive (0.81)	Active (0.66)	Active (0.94)	Inactive (0.88)
2	Bromadiolone	Inactive (0.53)	Inactive (0.74)	Active (0.83)	Active (0.91)	Inactive (0.78)
Phytoconstituents						
1	Quercetin	Inactive (0.70)	Inactive (0.88)	Active (0.57)	Active (0.82)	Inactive (0.93)
2	Kaempferol	Active (0.69)	Active (0.87)	Inactive (0.90)	Active (0.98)	Inactive (0.77)
3	Luteolin	Inactive (0.61)	Inactive (0.88)	Active (0.69)	Active (0.52)	Inactive (0.89)
4	Apigenin	Inactive (0.83)	Inactive (0.91)	Active (0.58)	Active (0.84)	Inactive (0.76)
5	Gallic Acid	Inactive (0.83)	Inactive (0.58)	Inactive (0.58)	Inactive (0.86)	Inactive (0.87)
6	Ellagic Acid	Inactive (0.73)	Inactive (0.65)	Active (0.52)	Inactive (0.62)	Active (0.52)
7	Hydroquinone	Inactive (0.80)	Inactive (0.73)	Active (0.50)	Active (0.62)	Inactive (0.67)
8	Scopoletin	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)
9	Coumarin	Inactive (0.74)	Active (0.56)	Inactive (0.93)	Active (0.81)	Inactive (0.78)
10	β-Sitosterol	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)
11	Lupeol	Inactive (0.59)	Active (0.61)	Inactive (0.52)	Active (0.65)	Inactive (0.57)
12	Stigmasterol	Inactive (0.81)	Inactive (0.61)	Inactive (0.71)	Inactive (0.61)	Inactive (0.66)
13	Myrtenol	Inactive (0.71)	Active (0.51)	Inactive (0.76)	Inactive (0.72)	Inactive (0.87)
14	Tea tree oil	Inactive (0.71)	Active (0.51)	Inactive (0.76)	Inactive (0.72)	Inactive (0.87)
15	Terpinen-4-ol	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)

ADME and Toxicity Evaluation of *Gliricidia Sepium* Phytoconstituents:

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties and toxicity profiles of *Gliricidia sepium* phytoconstituents were analyzed using computational approaches to determine their pharmacokinetic suitability for drug development (Table 3). The analyzed compounds include flavonoids, phenolic acids, and sterols, each exhibiting diverse solubility and permeability profiles. Flavonoids such as quercetin, kaempferol, and luteolin exhibit moderate lipophilicity (log P values: 1.31–1.99) and high topological polar surface area (TPSA > 100 Å²), suggesting good aqueous solubility and potential oral bioavailability. In contrast, sterols such as β-sitosterol and stigmasterol demonstrate high lipophilicity (log P > 5), indicating enhanced lipid solubility but potentially poor aqueous solubility, which may affect systemic absorption and distribution. Toxicity evaluations based on LD50 values and toxicity class assessments suggest that most compounds exhibit low to moderate toxicity (classes 3–6). Coumarin demonstrated the highest LD50 value (>70,000 mg/kg, Class 6), indicating a high margin of safety, whereas quercetin and gallic acid exhibited lower LD50 values (159 mg/kg and 225 mg/kg, respectively, Class 3), suggesting a need for further safety evaluations (Table 4). Molecular docking

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studies revealed that stigmasterol (-10.1 kcal/mol) and β-sitosterol (-9.8 kcal/mol) demonstrated strong binding affinities with key protein residues such as PHE230, GLY232, and TRP250. Kaempferol (-9.1 kcal/mol) and luteolin (-9.3 kcal/mol) also exhibited significant interactions, suggesting their potential as bioactive candidates for therapeutic applications.

Table 5 describes how different chemical constituents interact with various enzymes in the liver, called cytochrome P450 enzymes (CYP enzymes). These enzymes help break down drugs and other substances in the body. This shows which compounds are active and which are inactive. Warfarin and Bromadiolone are active in case of CYP2C9 and CYP2D6. Quercetin, Kaempferol, Apigenin, Scopoletin, Coumarin and β-Sitosterol are metabolized by CYP2D6. Kaempferol metabilized by CYP2C19. In conclusion, flavonoids, phenolic acids, and sterols from *Gliricidia sepium* exhibit promising ADME properties with favorable pharmacokinetic profiles and minimal toxicity risks. However, the potential hepatotoxicity and nephrotoxicity of certain compounds warrant further in vivo investigations. These results encourage more research into the plant compounds from *Gliricidia sepium* for use in medicines, especially for developing drugs that reduce inflammation, fight oxidation, and protect the heart.

Table 5: Prediction of in silico toxicity of chemical constituents from *Gliricidia sepium*

Sr. No	Name of Phyto-constituent	Hepato-Toxicity	Neuro-Toxicity	Nephro-Toxicity	Respiratory Toxicity	Cardio-Toxicity
Standard						
1	**Warfarin**	Inactive (0.72)	Inactive (0.81)	Active (0.66)	Active (0.94)	Inactive (0.88)
2	**Bromadiolone**	Inactive (0.53)	Inactive (0.74)	Active (0.83)	Active (0.91)	Inactive (0.78)
Phytoconstituent						
1	Quercetin	Inactive (0.7)	Inactive (0.88)	Active (0.57)	Active (0.82)	Inactive (0.93)
2	Kaempferol	Active (0.69)	Active (0.87)	Inactive (0.90)	Active (0.98)	Inactive (0.77)
3	Luteolin	Inactive (0.61)	Inactive (0.88)	Active (0.69)	Active (0.52)	Inactive (0.89)
4	Apigenin	Inactive (0.83)	Inactive (0.91)	Active (0.58)	Active (0.84)	Inactive (0.76)
5	Gallic Acid	Inactive (0.83)	Inactive (0.58)	Inactive (0.58)	Inactive (0.86)	Inactive (0.87)
6	Ellagic Acid	Inactive (0.73)	Inactive (0.65)	Active (0.52)	Inactive (0.62)	Active (0.52)
7	Hydroquinone	Inactive (0.80)	Inactive (0.73)	Active (0.50)	Active (0.62)	Inactive (0.67)
8	Scopoletin	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)
9	Coumarin	Inactive (0.74)	Active (0.56)	Inactive (0.93)	Active (0.81)	Inactive (0.78)
10	β-Sitosterol	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)
11	Lupeol	Inactive (0.59)	Active (0.61)	Inactive (0.52)	Active (0.65)	Inactive (0.57)
12	Stigmasterol	Inactive (0.81)	Inactive (0.61)	Inactive (0.71)	Inactive (0.61)	Inactive (0.66)
13	Myrtenol	Inactive (0.71)	Active (0.51)	Inactive (0.76)	Inactive (0.72)	Inactive (0.87)
14	Tea tree oil	Inactive (0.71)	Active (0.51)	Inactive (0.76)	Inactive (0.72)	Inactive (0.87)
15	Terpinen-4-ol	Inactive (0.85)	Inactive (0.51)	Inactive (0.92)	Active (0.79)	Inactive (0.83)

Toxicity Assessment of *Gliricidia sepium* Phytoconstituents

Toxicity assessment is a critical step in evaluating the safety profile of bioactive compounds, and computational methods provide an efficient alternative to traditional in vivo testing. Using the ProTox 3.0 server, toxicity predictions were performed for Gliricidia sepium phytoconstituents, focusing on five key toxicity endpoints: hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity. Warfarin and bromadiolone, used as standards, exhibited active nephrotoxicity and respiratory toxicity, with threshold values of 0.66 and 0.94 for warfarin and 0.83 and 0.91 for bromadiolone, respectively. Among the analyzed compounds, kaempferol exhibited hepatotoxic potential with a threshold value of 0.69,

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suggesting possible liver-related adverse effects. Several flavonoids, including quercetin, kaempferol, luteolin, and apigenin, displayed neurotoxic activity, indicating a potential risk for central nervous system toxicity. Notably, kaempferol showed the highest neurotoxicity threshold (0.87), necessitating further investigation into its neurological safety. Regarding nephrotoxicity, multiple compounds, including quercetin, luteolin, and apigenin, demonstrated active nephrotoxic potential, with values ranging from 0.50 to 0.69, suggesting a possible risk of kidney impairment. In terms of respiratory toxicity, flavonoids such as quercetin, luteolin, apigenin, and β-sitosterol exhibited activity, with apigenin showing the highest threshold (0.84), highlighting its potential pulmonary risks. Most compounds did not exhibit cardiotoxicity, with ellagic acid being the only exception (0.52 thresholds), indicating a low likelihood of cardiovascular toxicity among the tested compounds. Sterols such as stigmasterol and β-sitosterol remained inactive across all toxicity endpoints, supporting their potential as safe pharmaceutical or nutraceutical agents. These computer-based results highlight how important it is to check for toxicity when developing new drugs and suggest that while many parts of *Gliricidia sepium* could be useful for treatment, some compounds might need more testing to ensure they are safe for use in medicine.

4. Discussion

Natural bioactive compounds are increasingly studied for their therapeutic potential, necessitating in-depth pharmacological evaluation. This study looks at the health benefits of *Gliricidia sepium*, examining how it interacts at the molecular level, how it moves through the body, and its safety using computer-based methods. Molecular docking was performed utilizing CB-Dock2 to evaluate the binding affinity and molecular interactions of these compounds with the 8SSK protein. Warfarin and bromadiolone served as standard reference compounds.

The molecular docking analysis showed that stigmasterol (-10.1 kcal/mol) and β-sitosterol (-9.8 kcal/mol) had the strongest attraction to the 8SSK protein, meaning they interacted well with it. Kaempferol and luteolin also displayed significant binding affinities, with docking scores of -9.1 kcal/mol and -9.3 kcal/mol, respectively. Warfarin, used as a standard, showed a docking score of -8.8 kcal/mol, reinforcing the potential of these phytoconstituents as promising therapeutic agents.

The evaluation of ADME (absorption, distribution, metabolism, and excretion) properties highlighted diverse solubility and permeability profiles. Flavonoids such as quercetin, kaempferol, and luteolin exhibited moderate Log P values (0.50–1.99) and high Topological Polar Surface Area (TPSA > 100 Å²), indicating good aqueous solubility and potential oral bioavailability. In contrast, sterols such as β-sitosterol and stigmasterol demonstrated high Log P values (>5), suggesting enhanced lipid solubility but poor aqueous solubility, which may impact systemic absorption and distribution.

Toxicity predictions based on LD50 values and toxicity classifications revealed that most compounds exhibit low to moderate toxicity (classes 3–6). Coumarin had the highest LD50 value (over 70,000 mg/kg, Class 6), meaning it is very safe, while quercetin and gallic acid had lower LD50 values (159 mg/kg and 225 mg/kg, respectively, Class 3), which means they need more safety checks.

A comprehensive toxicity assessment was conducted using the ProTox 3.0 server, focusing on hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, and cardiotoxicity. Warfarin and bromadiolone were found to harm the kidneys and affect breathing, with specific values of 0.66 and 0.94 for warfarin and 0.83 and 0.91 for bromadiolone, respectively. Among the plant compounds, kaempferol showed a risk of liver damage with a threshold of 0.69, indicating it might cause liver-related problems. Quercetin, kaempferol, luteolin, and apigenin were found to have harmful effects on the nervous system, with kaempferol having the highest level of concern (0.87), which means it could pose risks to brain and nerve health.

Nephrotoxicity was identified in quercetin, luteolin, and apigenin, with threshold values ranging from 0.50 to 0.69, suggesting possible kidney impairment. In terms of respiratory toxicity, quercetin, luteolin, apigenin, and β-sitosterol showed some effects, with apigenin having the highest level (0.84), which points to possible risks for lung health. Most compounds did not exhibit cardiotoxicity, with ellagic acid being the only

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exception (0.52 thresholds), indicating a low likelihood of cardiovascular toxicity.

Sterols like stigmasterol and β-sitosterol showed no activity in any toxicity tests, suggesting they could be safe for use in medicines or health products. These computational findings emphasize the value of toxicity profiling in drug discovery and suggest that while many *Gliricidia sepium* constituents hold therapeutic promise, certain compounds require further preclinical validation to confirm their safety for pharmaceutical applications. The study emphasizes the prospective use of flavonoids, phenolic acids, and sterols from *Gliricidia sepium* as natural therapeutic agents, necessitating additional in vitro and in vivo studies to validate their efficacy and safety.

5. Conclusion

This study provides valuable insights into the pharmacological potential of bioactive compounds from *Gliricidia sepium*, particularly flavonoids, phenolic acids, and sterols. Molecular docking studies found that stigmasterol and β-sitosterol are good options because they strongly attach to the 8SSK protein. The ADME analysis showed that flavonoids are likely to dissolve well and be absorbed by the body, while sterols are very fat-loving, which could affect how well they are taken in by the system. Toxicity assessments revealed that most compounds exhibited low to moderate toxicity, with coumarin demonstrating the highest safety margin. However, kaempferol displayed potential hepatotoxicity, and quercetin and apigenin exhibited nephrotoxic activity, which calls for further safety evaluations. Despite these concerns, sterols such as β-sitosterol and stigmasterol remained inactive across all toxicity endpoints, reinforcing their potential as safe therapeutic agents. Overall, the findings suggest that *Gliricidia sepium* constituents hold significant promise for pharmaceutical applications. However, further in vitro and in vivo studies are necessary to confirm their efficacy and safety, paving the way for their potential development as therapeutic agents.

References

1. Brogi, S. (2019). Computational approaches for drug discovery. Molecules, 24(17), 3061.
2. Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific reports, 7(1), 42717.
3. Drwal, M. N., Banerjee, P., Dunkel, M., Wettig, M. R., & Preissner, R. (2014). ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic acids research, 42(W1), W53-W58.
4. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, 31(2), 455-461.
5. Wang, Y., Xiao, J., Suzek, T. O., Zhang, J., Wang, J., & Bryant, S. H. (2009). PubChem: a public information system for analyzing bioactivities of small molecules. Nucleic acids research, 37(suppl_2), W623-W633.
6. Lipinski, C. A. (2004). Lead-and drug-like compounds: the rule-of-five revolution. Drug discovery today: Technologies, 1(4), 337-341.
7. Kapetanovic, I. (2008). Computer-aided drug discovery and development (CADDD): in silico-chemico-biological approach. Chemico-biological interactions, 171(2), 165-176.
8. Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., ... & Woolsey, J. (2006). DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic acids research, 34(suppl_1), D668-D672.
9. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry, 30(16), 2785-2791.
10. Egan, W. J., Merz, K. M., & Baldwin, J. J. (2000). Prediction of drug absorption using multivariate statistics. Journal of medicinal chemistry, 43(21), 3867-3877.
11. Ali, A., Mir, G. J., Ayaz, A., Maqbool, I., Ahmad, S. B., Mushtaq, S., ... & Rehman, M. U. (2023). In silico analysis and molecular docking studies of natural compounds of Withania somnifera against bovine NLRP9. Journal of Molecular Modeling, 29(6), 171.
Mahmud, S., Paul, G. K., Afroze, M., Islam, S., Gupt, S. B. R., Razu, M. H., ... & Simal-Gandara, J.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 1757-1767 | ISSN:2251-6727

(2021). Efficacy of phytochemicals derived from Avicennia officinalis for the management of COVID-19: a combined in silico and biochemical study. Molecules, 26(8), 2210.

12. Islam, S., Salekeen, R., & Ashraf, A. (2024). Computational screening of natural MtbDXR inhibitors for novel anti-tuberculosis compound discovery. Journal of Biomolecular Structure and Dynamics, 42(7), 3593-3603.
13. Malla, B. A., Ali, A., Maqbool, I., Dar, N. A., Ahmad, S. B., Alsaffar, R. M., & Rehman, M. U. (2023). Insights into molecular docking and dynamics to reveal therapeutic potential of natural compounds against P53 protein. Journal of Biomolecular Structure and Dynamics, 41(18), 8762-8781.
14. Khan, J., Sakib, S. A., Mahmud, S., Khan, Z., Islam, M. N., Sakib, M. A., ... & Simal-Gandara, J. (2022). Identification of potential phytochemicals from Citrus limon against main protease of SARS-CoV-2: Molecular docking, molecular dynamic simulations and quantum computations. Journal of Biomolecular Structure and Dynamics, 40(21), 10741-10752.
15. Surana, K. R., & Mahajan, S. K. (2022). In silico study of chromane ring compound rubranonoside from Plumeria rubra as anticancer potential. Trends in Sciences, 19(24), 3305-3305.
16. Alsaffar, R. M., Ali, A., Rashid, S. M., Ahmad, S. B., Alkholifi, F. K., Kawoosa, M. S., ... & Rehman, M. U. (2023). Zerumbone protects rats from collagen-induced arthritis by inhibiting oxidative outbursts and inflammatory cytokine levels. Acs Omega, 8(3), 2982-2991.
17. Bhardwaj, V. K., Singh, R., Sharma, J., Rajendran, V., Purohit, R., & Kumar, S. (2021). Identification of bioactive molecules from tea plant as SARS-CoV-2 main protease inhibitors. Journal of Biomolecular Structure and Dynamics, 39(10), 3449-3458.
18. Tong, X., Zhang, H., & Timmermann, B. N. (2011). Chlorinated withanolides from Withania somnifera. Phytochemistry letters, 4(4), 411-414.
19. Vuignier, K., Schappler, J., Veuthey, J. L., Carrupt, P. A., & Martel, S. (2010). Drug–protein binding: a critical review of analytical tools. Analytical and bioanalytical chemistry, 398, 53-66.
20. Jain, J., Narayanan, V., Chaturvedi, S., Pai, S., & Sunil, S. (2018). In vivo evaluation of withania somnifera–based Indian traditional formulation (Amukkara choornam), against chikungunya virus–induced morbidity and arthralgia. Journal of evidence-based integrative medicine, 23, 2156587218757661.
21. Cai, Z., Zhang, G., Tang, B., Liu, Y., Fu, X., & Zhang, X. (2015). Promising anti-influenza properties of active constituent of Withania somnifera ayurvedic herb in targeting neuraminidase of H1N1 influenza: computational study. Cell biochemistry and biophysics, 72, 727-739.
22. Ganguly, B., Umapathi, V., & Rastogi, S. K. (2018). Nitric oxide induced by Indian ginseng root extract inhibits Infectious Bursal Disease virus in chicken embryo fibroblasts in vitro. Journal of Animal Science and Technology, 60(1), 2.
23. Surana, K. R., Jadhav, P. S., Shewale, H. S., Wagh, D. B., Mahajan, S. K., & Musale, J. V. (2024). Insilico and Biological Evaluation of Anti-Inflammatory Activity of synthesized Benzimidazoles Derivatives. Biosciences Biotechnology Research Asia, 20(3), 1241-1253.
24. Aher, S. N., Sonawane, S. N., Sonawane, P. R., Surana, K. R., Mahajan, S. K., & Patil, D. M. (2024). Insilico Drug Design, Synthesis and Evaluation of Anti-inflammatory Activity Pyrimidine Analogue. Biosciences Biotechnology Research Asia, 21(2), 741-753.