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Molecular Docking and Virtual Screening Analysis of *Centella asiatica* Constituents as Antiulcer Agents

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Abstract: One of the most remarkable natural herbal items is *Centella asiatica*'s chemical components. The corresponding chemical components were extracted from the *Centella asiatica* species, which was found in tropical Australia, Asia, Africa, and the western Pacific Ocean islands. The chemical components of *Centella asiatica* have distinctive pentacyclic triterpenoid analogs, and they have a variety of biological effects, including antimicrobial, anticancer, wound-healing, neuroprotective, anti-inflammatory, insecticidal, and antioxidant effects. The primary aim of the present study is to dock the chosen chemical constituents of *Centella asiatica* on to the protein 2ZXB and compare the results to those of omeprazole as a standard drug. For the docking test against protein gastric H^+/K^+ ATPase (PDB code: 2XZB), chemical constituents of *Centella asiatica* were used for the currentwork.

Modern drug design, virtual screening, and molecular docking are commonly used to understand drug-receptor interactions. PyRx, discovery studio visualizer (DSV), and Swiss dock application were used to carry out the virtual screening and molecular docking analysis.All 46 constituents of *Centella asiatica* docking scores were discovered to range between -7.4 to -17 kcal/mol. At the protein active site compound Asiaticoside-B interact with amino acids such as Asparagine (ASN A 138), Aspartic acid (ASP A 137), Tyrosine (TYR A 799, TYR A 925 & TYR A 928), Isoleucine (ILE A 814), Glycine (GLY A 812), Cysteine (CYS A 813). The binding site, activity and affinity score of numerous chemical constituents of *Centella asiatica* have been identified. According to the findings, *Centella asiatica* constituents exhibited antiulcer activity.

Keywords: ADME, H^+/K^+ ATPase, Asiaticoside-B, *Centella asiatica*, Docking, Antiulcer agents

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Introduction

The ethnic medicinal herb *Centella asiatica* (Linn) is employed by numerous ancient societies and tribal groups throughout the world. It is one of the regional herbs that is said to have a variety of physiological benefits and plays a significant role in the traditional medical system as a tonic for leprosy and skin illnesses (Saurabh *et al*., 2021). *Centella asiatica* chemical components, which include pentacyclic triterpenoids and trisaccharide analogs, have a variety of bioactivities, including antimicrobial activity (Roy *et al*., 2023), anticancer activity (Hamid IS*et al.,* 2016), wound healing activity (Khotimah *et al*., 2021), neuroprotective activity (Gray *et al*., 2018), immunomodulatory activity (Al-Mamun *et al*., 2023), anti-inflammatory activity (Thakral *et al*., 2023), hepatoprotective activity (Awuchi *et al*., 2023), insecticidal activity (Noor *et al*., 2017), and antioxidant activity (Silva *et al*., 2013). Omeprazole was employed in this trial as the standard anti-ulcer medication. It is a proton pump inhibitor that has been successfully treating diseases associated with stomach acid secretion for around 15 years (Huang *et al*., 2022).

Centella asiatica, a plant belonging to the Apiaceae family, is also referred to as gotu kola, kodavan, Indian pennywort, and Asiatic pennywort. The globe over, it is a little perennial creeping plant that thrives in damp tropical and subtropical climates. The plants have long petioles that are grouped at the stem nodes and bear scalloped-edged shovel- or spade-shaped leaves. The inconsequential green or pinkish-white blooms are borne in numerous umbels, and the 3-5 mm long pumpkin-shaped seeds are nutlets. *Centella asiatica* has been reported to contain both isoprenoids (sesquiterpenes, plant sterols, pentacyclic triterpenoids, and saponins) and phenylpropanoid derivatives (eugenol derivatives, caffeoylquinic acids, and flavonoids).

The herb or its by-products are being used in viable, topical, and oral medications throughout the world. Internal preparations of the herb were used to treat simple dysentery, stomach ulcers, and syphilitic lesions while topically applied formulations were used to treat infectious skin illnesses, speed up the healing of skin ulcers, and treat injuries (Zheng *et al*., 2016; Sharma *et al*., 2017; Narayanan *et al*., 2018; Sharifi-Rad *et al*., 2018; Kuna *et al*., 2019; Rani *et al*., 2019; Sahoo 2019; Kambale *et al*., 2022; Awuchi *et al*., 2023; Al-Mamun *et al*., 2023).

A potential target for the components of *Centella asiatica* in their fight against stomach ulcers is the protein 2XZB. Recently, there has been a lot of interest in inhibiting H^+/K^+ ATPase as a way to regulate gastric pH due to the discovery that it is the main gastric proton pump. (Gadekar *et al*., 2010). Protein 2XZB is also known to be a proton pump specifically from pig gastric. As in the process of HCl production, the exchange of H^+ with K^+ is the last step, as these H+ ions combine with Cl^- ion in the stomach. Thus blockage of this receptor inhibits the exchange of ions and thus no HCl production. Thus this receptor was specifically chosen to obtain anti-ulcer activity through the mechanism of proton pump inhibition.

Based on molecular docking analysis and physiochemical parameters, a group of 46 *Centella asiatica* analogues that exhibit inhibitory activity against H+, and K^+-ATPase were examined. It was discovered that in addition to binding affinity, H-bond donor and acceptor fields were crucial for the H^+, and K^+-ATPase inhibition activity of these compounds. In the current research, we conducted an *in silico* analysis on a brand-new group of chemicals, specifically a group of *Centella asiatica* compounds that serve as PPI inhibitors. Docking investigations on these chemicals have not been reported so far. The primary goal of this study is to present the physicochemical characteristics that control the activity of these chemicals. We have reported some new compounds from the series that might have better activity using the Swiss dock model. These novel compounds have undergone docking research, and the outcomes have been contrasted with those of licensed

compounds. These compounds' ADME/T characteristics have also been investigated and verified using Lipinski criteria.

Since H^+/K^+ ATPase is the main proton pump in the stomach, inhibiting it to regulate stomach pH has garnered a lot of attention. This is especially true since the *Centella asiatica* class of anti-ulcer drugs was discovered recently. One of the earliest known inhibitors of the stomach proton pump was omeprazole, which was more effective than picoprazole.

Materials and Methods

Molecular docking studies

PyRx software 0.8, which is free and open-source software that includes AutoDock4 and AutoDock Vina, was used to calculate docking interactions. Understanding how various ligands interact with the receptor's active site was the aim of the research. The entire molecular docking process was carried out as follow:

Molecular docking protocol

PyRx 0.8 software was used to determine the manner of binding and interaction of the chosen target proteins with specific ligands. A probable shape and orientation for the ligand at the active site were obtained using docking. A PDBQT file containing the structure of proteins with Hydrogens in all polar residues was created when the protein was fed into the PyRx software program. By creating a grid box with dimensions, the docking site on the protein-ligand was identified. The lowest binding scores led to the best confirmation being selected. Pymol software was used to build the complex of ligand-protein interaction.

Selection of Data Set and Preparation of Ligand

The 45 compounds listed in Table 1 were chosen from a series of novel structures of Asiaticoside B, Madecassic acid, Brahminoside B, Castillicetin, Centillosaponin B, Centillosaponin C, Asiaticoside E, Asiaticoside F, Asiaticoside G, and Centelloside E (SDF File), which were taken from the US National Library of Medicine PubChem (https://pubchem.ncbi.nlm.nih.gov/#query=centella%20asiatica). The Universal Force Field (UFF) carried out energy minimization. The chemical makeup of *Centella asiatica* analogs and the structures of model drugs are illustrated in Table 1 and Figure 1.

Preparation of Protein Molecule

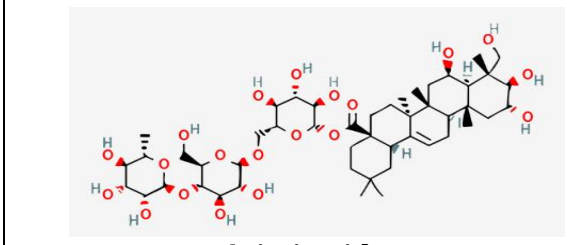
The newly discovered protein gastric H^+/K^+ATP6ase structure file was obtained from the RCSB Protein Data Bank and stored in the PDB format (PDB ID: 2XZB) (https://www.rcsb.org/structure/2XZB) (Fig. 2). As soon as the PDB file for protein gastric H^+/K^+ATP6ase is loaded in the discovery studio client, the crystal structure of the ligand-binding domain of the 2XZB protein is displayed in a 3D window with a bound ligand and crystallographic fluids. The water molecules in question are not visible in the Hierarchy view. The protein structure has been cleansed and is now exposed to polar hydrogen atoms. To save the file in the PDB structure, the related ligand has been removed, and the ligand's binding site can be predicted from the current pose.

The protein preparation process included a few phases:

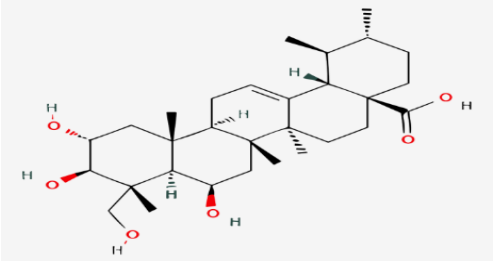
- ✓ Orders for bond assignments
- ✓ Atoms of polar hydrogen are added.
- ✓ Removal of metal bond.
- ✓ At pH 7.0, hydrogen bonding was optimized.
- ✓ Reorienting the water, -OH group and amino acids resolved overlapping problems in the hydrogen bond network.
- ✓ By making a decision in "Review and modify" tab, only the necessary portion of the protein under research was maintained, and the remainder was removed.
- ✓ The co-crystallized ligand's potential protonation states were then generated by selecting the generate states option. To address any issues, the ligand's structure was examined.

Table 1: Chemical Constituents of *Centella asiatica* analogs and their docking score

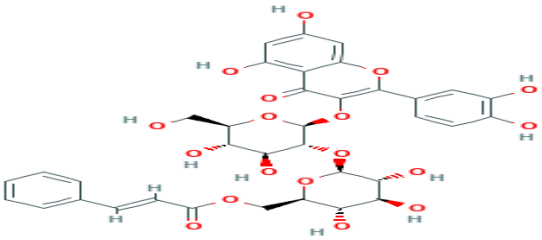
S. No.	Compound	Compound PubChem Id	Docking Score
1	Asiaticoside B	91618002	-17
2	Madecassic acid	51531966	-14.6
3	Brahminoside	101504860	-10.4
4	Castillicetin	102394640	-10.3
5	Centellasaponin B	101140416	-10.3
6	Centellasaponin C	101103167	-10.0
7	Asiaticoside E	102212085	-9.9
8	Asiaticoside F	53317001	-9.9
9	Asiaticoside G	53320941	-9.9
10	Centelloside E	101568838	-9.9
11	Scheffuroside F	101675278	-9.9
12	Rutin	5280805	-9.9
13	Asiaticoside	108062	-9.8
14	Naringin	442428	-9.8
15	Asiaticoside D	102212084	-9.7
16	Centellasaponin D	101103168	-9.6
17	Centelloside D	56964357	-9.6
18	Madecassoside	45356919	-9.4
19	1,5-dicaffeoylquinic acid	122685	-9.3
20	Centellasaponin A	21633803	-9.3
21	Campesterol	173183	-9.2
22	Corosolic acid	6918774	-9.1
23	Ursolic acid	64945	-8.9
24	Asiaticoside C	101103169	-8.9
25	Catechin	9064	-8.8
26	Brahmic acid	73412	-8.8
27	3-epimaslinic acid	25564831	-8.8
28	Asiatic acid	119034	-8.7
29	Arjunolic acid	73641	-8.7
30	Cryptochlorogenic acid	9798666	-8.7
31	Centellasapogenol	15508087	-8.5
32	3,4-Dicaffeoylquinic acid	5281780	-8.5
33	Terminolic acid	12314613	-8.4
34	1,3-Dicaffeoylquinic acid	6474640	-8.4
35	Sitosterol	222284	-8.3
36	Bicyclogermacrene	5315347	-8.2
37	Castilliferol	10526707	-8.2
38	Patuletin	5281678	-8.2
39	Quetcetin	5280343	-7.9
40	Epicatechin	72276	-7.7
41	Germacrene B	5281519	-7.7
42	Chlorogenic acid	1794427	-7.6
43	Stigmasterol	5280794	-7.6
44	Pomolic acid	382831	-7.6
45	α -Humulene	5281520	-7.4
46	Omeprazole	4594	-7.2



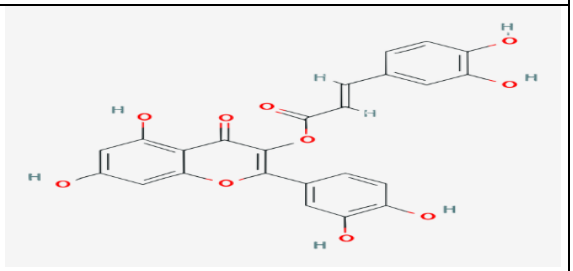
Asiaticoside B



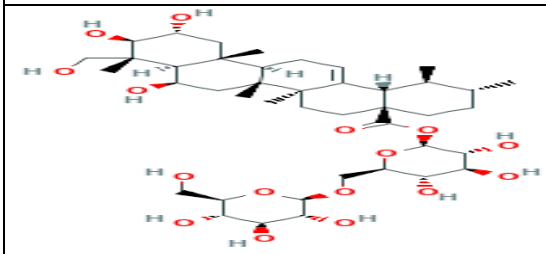
Madecassic acid



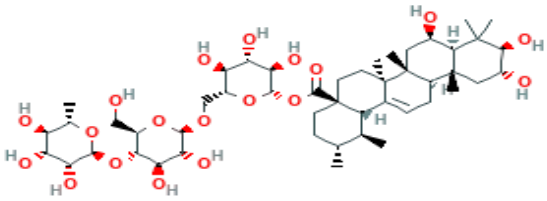
Brahminoside B



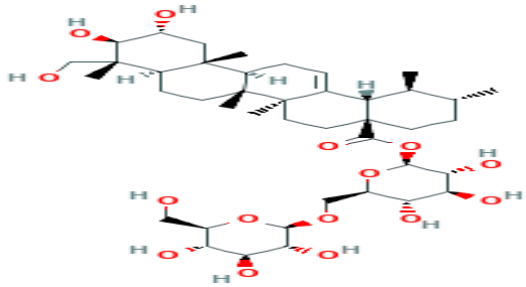
Castillicetin



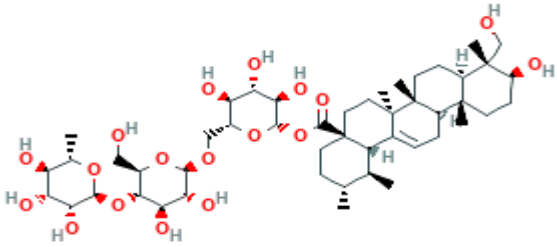
Centillosaponin B



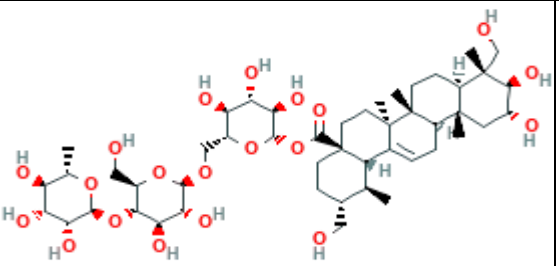
Centillosaponin C



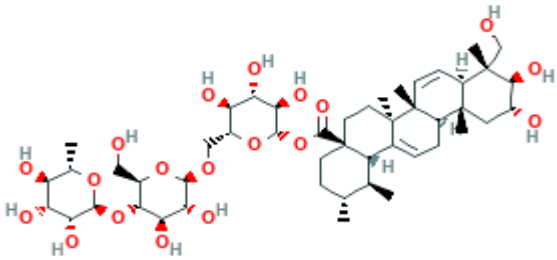
Asiaticoside E



Asiaticoside F



Asiaticoside G



Centelloside E

Fig. 1: Selected chemical compounds of *Centella asiatica* analogs.

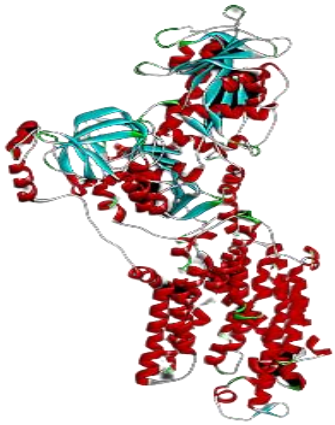


Fig. 2: Gastric H^+/K^+ATPase (PDB ID: 2XZB).

- ✓ Finally, the structure was refined with the aid of constrained minimization (Kumar *et al*., 2018).

Generation of Receptor Grid

The active site receptor grid was located using glide docking, a method that used a ligand linked to the X-ray crystal structure of a protein. Grid-based molecular docking has been employed to assist the ligands in binding in a few possible conformations. Glide molecular docking has also employed the scaling aspect of 0.25, the partial charge cutoff of 1 for the Van Der Waals radius, and other parameters (Sharma *et al*., 2016).

Glide molecular docking study

Following the preparation of the ligands, protein, and grid on the biological target's catalytic site, molecular docking was carried out. Glide molecular docking makes use of a method of computational modeling to evaluate binding postures. An empirical scoring formula called the G-score is the result of the more current glide systematic approach, which produces rapid, distinctive molecular docking. The ligand-protein interaction energies that make up the calculated binding energy are represented in Kcal/mol. It also calculated the outcome's root mean square deviation (RMSD), internal energy, H-bond, stacking interactions, and desolvation energy. The XP visualizer was employed to carefully examine the ligand-protein interaction. Most of the chosen ligands, including the reference molecules, were docked with the X-ray crystal structure using glide (Mehta *et al*., 2021, 2022).

Evaluation of In Silico Study

Once the docking score was established, the outcomes of the docking study were evaluated using the conformation of the ligand-protein interaction. The compounds that bound to the target receptor protein most effectively had the best interaction profiles and highest dock scores.

Results

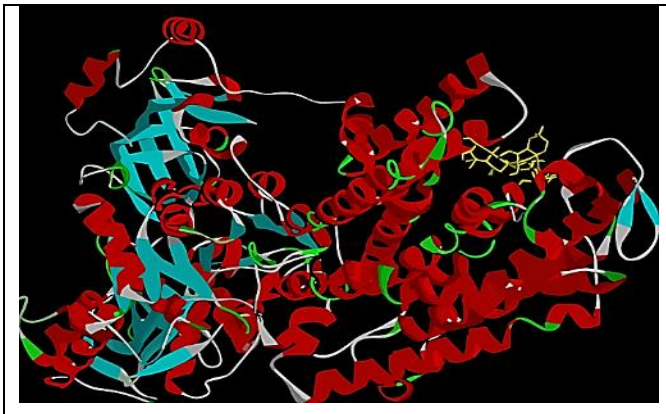
The docking simulation techniques were carried out with *Centella asiatica* constituents on H^+/K^+ATPase as the protein target by using PyRx software. The comparison of the fitness function score and ligand binding location were the two parameters that were utilized to determine which protein was best docked. The pharmacological activity that the docking score predicts indicates the range of binding energy that is needed to attach a ligand to the receptor. Additionally, it helps to improve the interaction between ligand receptors.

Molecular docking analysis

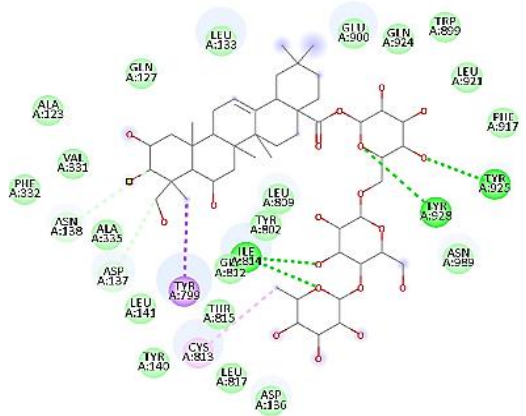
A computational ligand-protein docking method was used to analyze the structural complex of the 2XZB (Protein) with *Centella asiatica* constituents like Asiaticoside B, Madecassic acid, Brahminoside B, Castillicetin, Centellosaponin B, Centellosaponin C, Asiaticoside E, Asiaticoside F, Asiaticoside G, Centelloside E (Ligands) (Table 2; Fig. 3). The

Table 2: List of amino acid binding with H^+/K^+ATPase

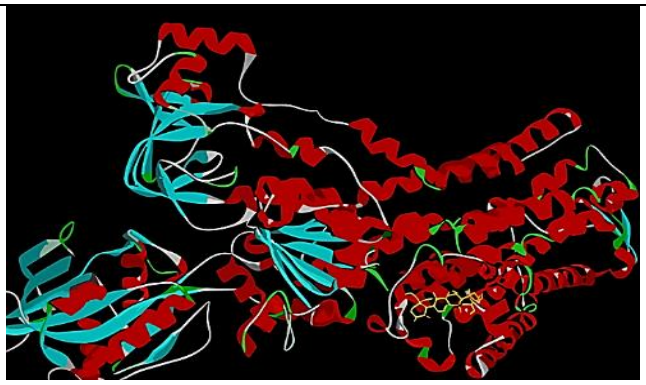
S. No.	Ligand	Interacting amino acids	Number of amino acids
1	Asiaticoside B	Asparagine(A:138), Asparticacid(A:137),Tyrosine(A:925) (A:928) (A:799), Isoleucine(A:814), Glycine(A:812), Cysteine(A:813)	8
2	Madecassicacid	Tyrosine(A:1033), Arginine(A:844)	2
3	Brahminoside B	Tryptophan(A:899), Tyrosine(A:928) (A:799), Leucine(A:809), Threonine(A:134), Alanine(A:335), Cysteine(A:813), Asparagine(A:138) (A:989)	9
4	Castillicetin	Leucine(A:370), Cysteine(A:100)	2
5	Centellosaponin B	Glutamic acid(A:795), Cysteine(A:813), Valine(A:331), Asparagine(A:138), Asparticacid(A:137),Glutamine(A:127), Isoleucine(A:814)	7
6	Centellosaponin C	Tyrosine(A:1032) (A:1033), Arginine(A:852) (A:949) (A:775) (A:844) (A:850), Lysine(A:782), Proline(A:845), Glutamic acid(A:374)	10
7	Asiaticoside E	Tyrosine(A:828), Alanine, Aspartic acid(A:137) (A:136)(A:335), Asparagine(A:138)	5
8	Asiaticoside F	Aspartic acid(A:779) (A:851), Arginine(A:949) (A:850), Lysine(A:782), Tyrosine(A:1033), Glutamic acid(A:856)	7
9	Asiaticoside G	Tryptophan(A:899), Aspartic acid(A:137), Asparagine(A:138)	3
10	Centelloside E	Glutamine(A:924)	1
11	Omeprazole	Arginine(A:775) (A:846), Asparticacid(A:851), Glutamic acid(A:374), Leucine(A:782), Tyrosine(A:1032) (A:1033)	7



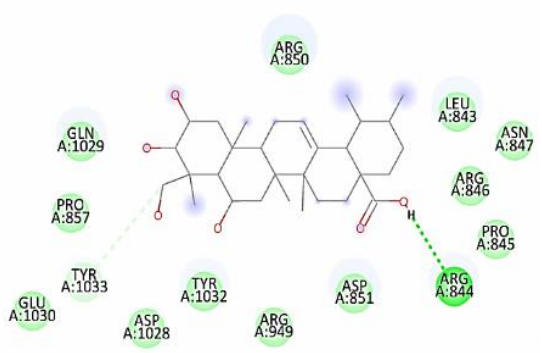
Asiaticoside Binteractionwith2XZB



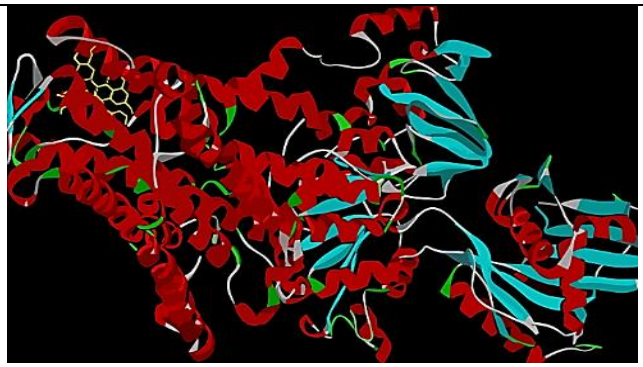
Target active site of Asiaticoside B



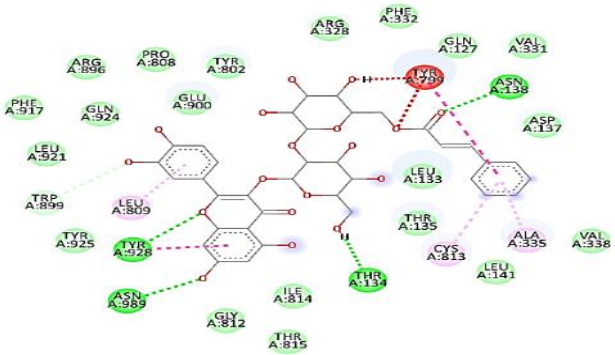
Madecassic acid interactionwith2XZB



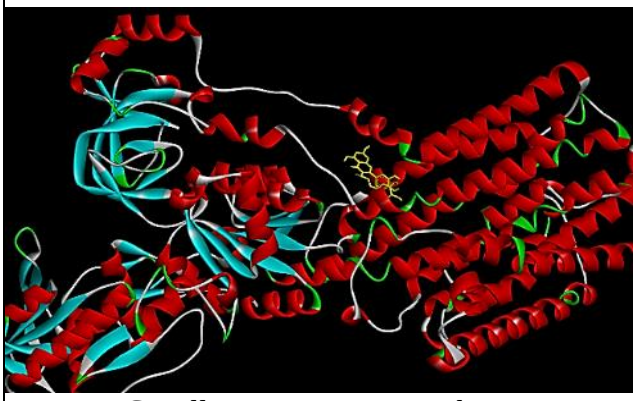
Target active site of Madecassic acid



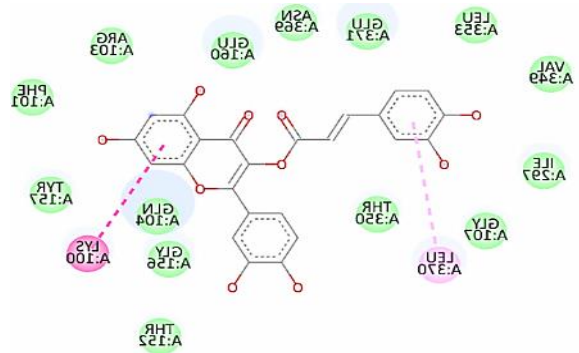
Brahminoside Binteractionwith2XZB



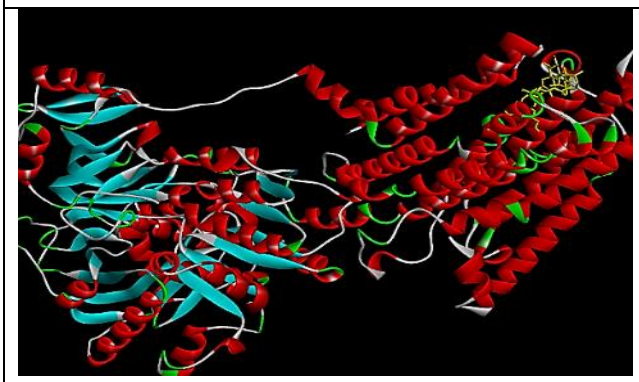
Target active site of Brahminoside B



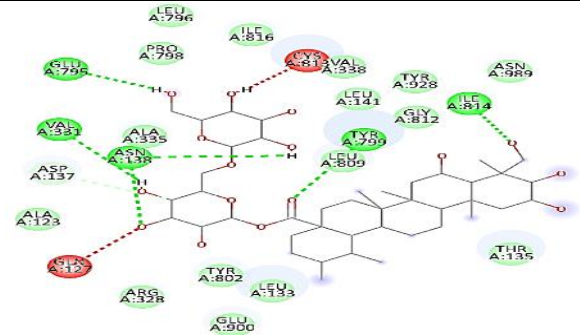
Castillicetininteractionwith2XZB



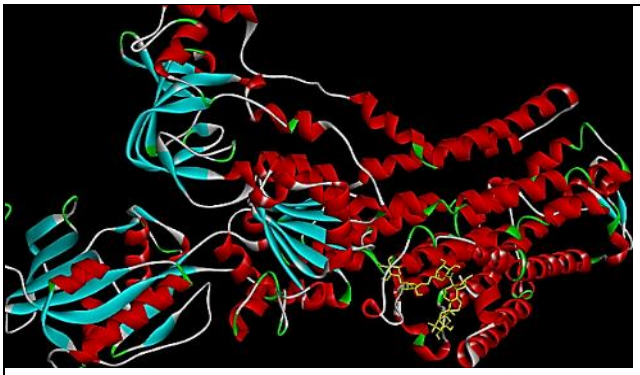
Target active site of Castillicetin



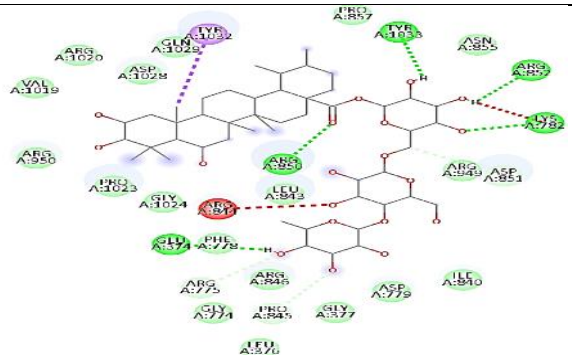
Centellosaponin B interactionwith2XZB



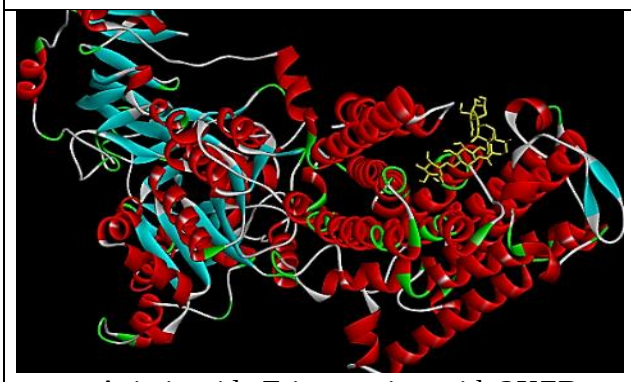
Target active site of Centellosaponin B



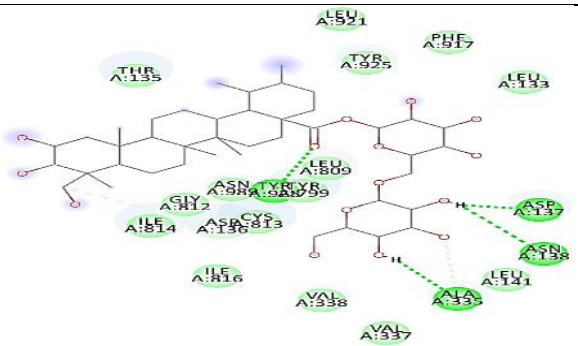
Centellosaponin C interaction with 2XZB



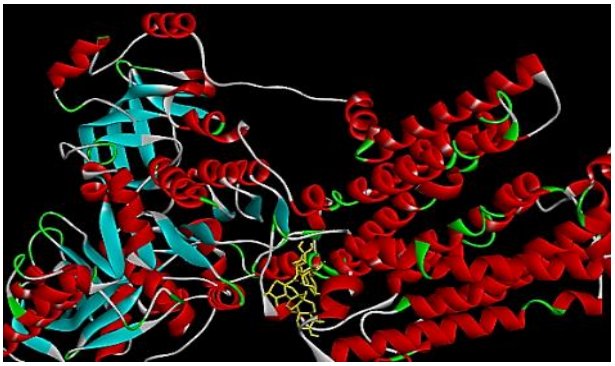
Target active site of Centellosaponin C



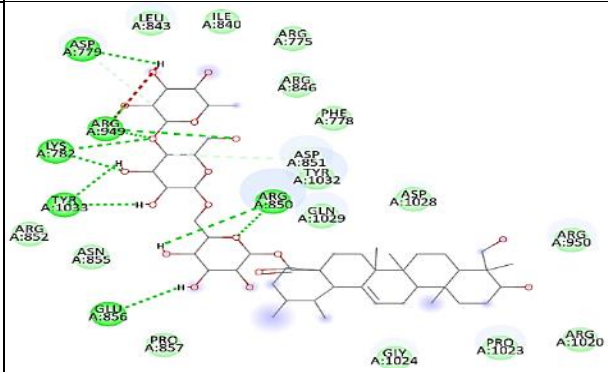
Asiaticoside E interaction with 2XZB



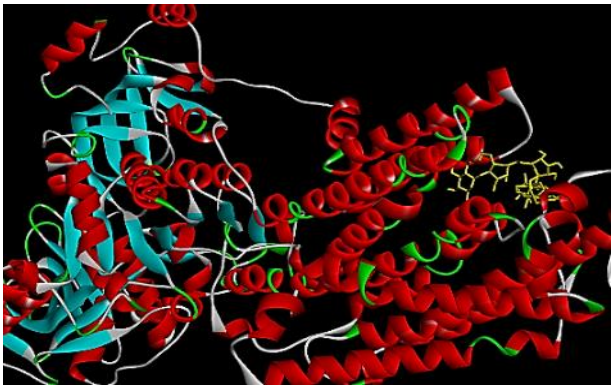
Target active site of Asiaticoside E



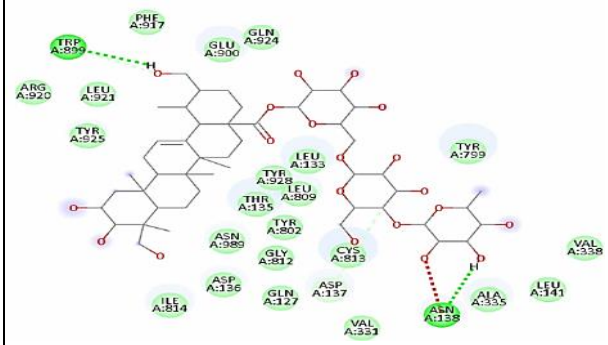
Asiaticoside F interaction with 2XZB



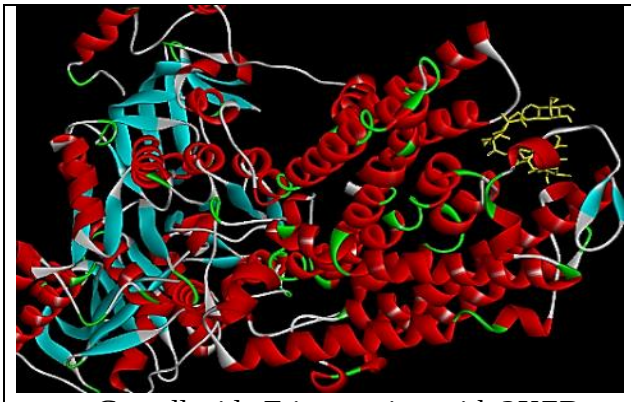
Target active site of Asiaticoside F



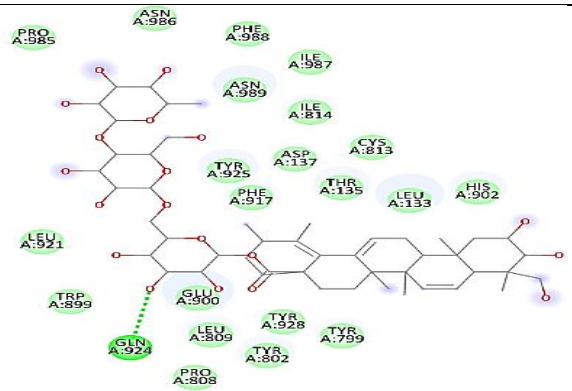
Asiaticoside G interaction with 2XZB



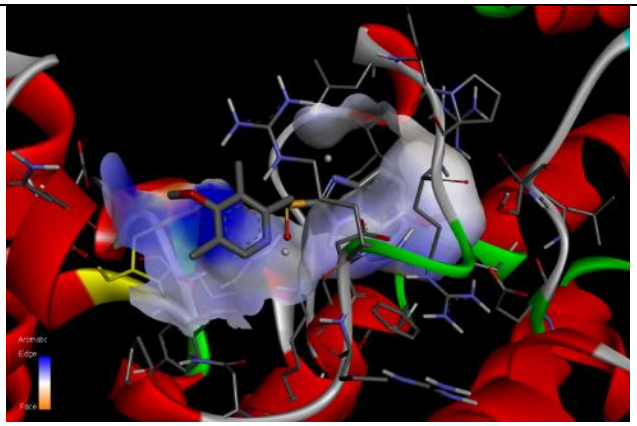
Target active site of Asiaticoside G



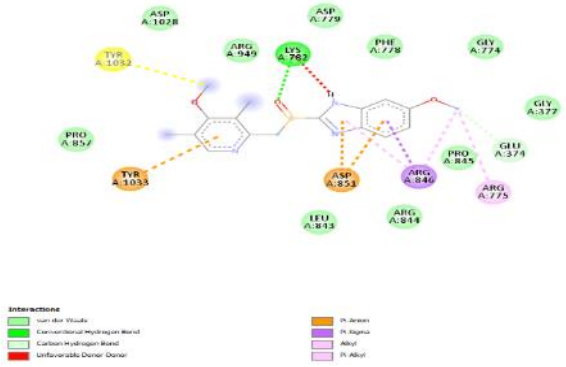
Centelloside E interaction with 2XZB



Target active site of Centelloside E



Omeprazole interaction with 2XZB



Target active site of Omeprazole

Table 3: Physiochemical and Pharmacokinetics Properties of Compound Asiaticoside B

Formula	$C_{48}H_{78}O_{20}$
Molecularweight	975.12g/mol
Num.heavyatoms	68
Num.aromatic.heavyatoms	0
FractionCsp3	0.94
Num.H-bondacceptors	20
Num.H-bonddonors	13
MolarRefractivity	235.72
TPSA	335.44 $Å^2$
Num.rotatablebonds	10
GIabsorption	Low
BBBpermeant	No
P-gpsubstrate	Yes
CYP1A2inhibitor	No
CYP2C19inhibitor	No
CYP2C9inhibitor	No
CYP2D6inhibitor	No
CYP3A4inhibitor	No
LogKp(skinpermeation)	-13.03cm/s

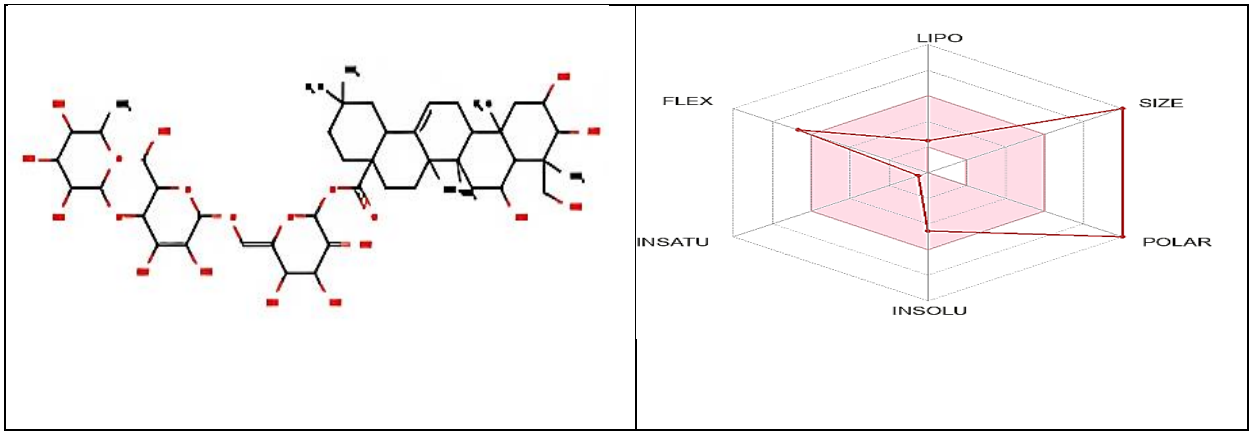


Fig. 4: Bioavailability radar chart for isolated Asiaticoside B. The pink zone represents the physicochemical space for oral bioavailability, and the red line represents the oral bioavailability properties.

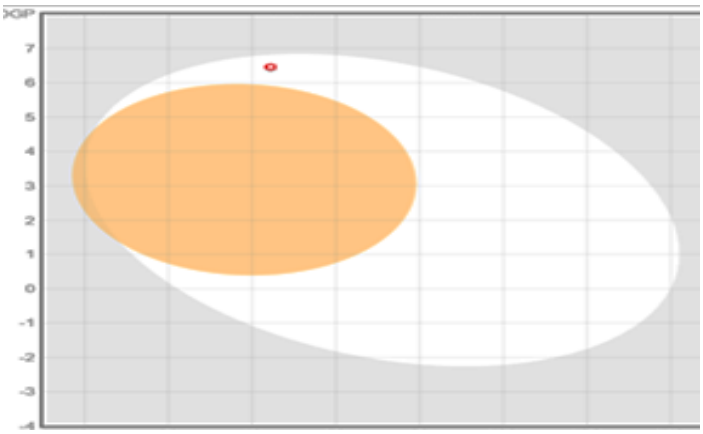


Fig. 5: Predicted BOILED-Egg plot from *Swiss ADME* online web tool for SWISS DOCK.

docking process was finally completed by PyRx using the Auto-dock vina option based on the scoring algorithm. A "grid point" value is assigned to the interaction energy of the elements of *Centella asiatica* with 2XZB. At each stage of the simulation, the ligand and protein's interaction energy was calculated using atomic affinity potentials generated on a grid, while the other parameters were left at their default values.

ADME Prediction

Physiochemical, Pharmacokinetics, and drug-likeness calculation of several physiochemical features and pharmacokinetics signifiers were predicted through the online web tool SWISS DOCK (Table 3; Figs. 4, 5).

The chances of the created chemicals successfully interacting with their target organisms are high. With the help of Swiss ADME tools (skin permeation), calculations were done for intestinal absorption (per cent absorbed) and permeability of the Log Kp. Every chosen compound interacts with cytochromes as a substrate or as an inhibitor. The compounds are likely liver-toxic, thus more research will be necessary to determine the hepatotoxic dose level. Because all of the suggested compounds display

Table 4: Drug likeness analysis of designed *Centella asiatica* Constituents (1a-2t)

Comp Code	Physicochemical Properties						Lipinski's Rule	%Abs[d]	N viol[e]	N rot[f]
	Ligand	MW[a]	TPSA[b]	logP[c]	H_A	H_D				
1a	Asiaticoside B	975.12	335.44	1.91	13	20	No	89.42	10	3
1b	Madecassic acid	504.70	118.22	3.20	5	6	Yes	89.42	2	1
1c	Brahminoside	756.66	295.73	3.02	10	18	No	89.42	11	3
1d	Castillicetin	464.38	177.89	1.83	6	10	Yes	89.42	5	1
1e	Centellasaponin B	828.98	276.52	3.66	11	16	No	86.23	8	3
1f	Centellasaponin C	959.12	315.21	2.60	12	19	No	86.23	9	3
1g	Asiaticoside E	812.98	256.29	2.70	10	15	No	86.23	8	3
1h	Asiaticoside F	943.12	294.98	2.09	11	18	No	89.42	10	3
1i	Asiaticoside G	975.12	335.44	3.99	13	20	No	89.42	11	3
1j	Centelloside E	975.11	315.21	3.60	12	19	No	89.42	10	3
1k	Scheffuroside F	959.12	315.21	3.05	12	19	No	73.61	10	3
1l	Rutin	610.52	269.43	1.58	10	16	No	73.61	6	3
1m	Asiaticoside	959.12	315.21	3.05	12	19	No	73.61	10	3
1n	Naringin	580.53	225.06	2.38	8	14	No	82.44	6	3
1o	Asiaticoside D	943.12	294.98	2.96	11	18	No	82.44	9	3
1p	Centellasaponin D	959.12	315.21	3.73	19	12	No	0.25	3	10
1q	Centelloside D	829.98	276.52	2.60	16	11	No	13.60	3	8
1r	Madecassoside	975.12	335.44	1.87	20	13	No	-6.72	3	10
1s	1,5dicaffeoylquinic acid	516.45	211.28	1.11	12	7	No	36.10	3	9
1t	Centellasaponin A	959.12	315.21	4.01	19	12	No	0.25	3	10
1u	Campesterol	400.68	20.23	4.92	1	1	Yes	102.02	1	5
1v	Corosolic acid	472.70	77.76	3.39	4	3	Yes	82.17	1	1
1w	Ursolic acid	456.70	57.53	3.71	3	2	Yes	89.15	1	1
1x	Asiaticoside C	1001.1	321.28	4.06	20	11	No	-1.84	3	12
1y	Catechin	290.27	110.38	1.47	6	5	Yes	70.91	0	1
1z	Brahmic acid	504.70	118.22	3.20	5	6	Yes	68.21	2	1
2a	3-epimaslinic acid	472.70	77.76	3.55	3	4	Yes	82.17	1	1
2b	Asiatic acid	488.70	97.99	3.20	4	5	Yes	75.19	2	0
2c	Arjunolic acid	488.70	97.99	3.23	4	5	Yes	75.19	2	0
2d	Cryptochlorogenic acid	354.31	164.75	1.01	6	9	Yes	52.16	5	1
2e	Centellasapogenol	488.70	97.99	1.86	4	5	Yes	75.19	2	0
2f	3,4-Dicaffeoylquinic acid	516.45	211.28	1.25	7	12	No	36.10	9	3
2g	Terminolic acid	504.70	118.22	2.25	5	6	Yes	68.21	2	1
2h	1,3-Dicaffeoylquinic acid	516.45	211.28	1.11	7	12	No	36.10	9	3
2i	Sitosterol	414.71	20.23	4.79	1	1	Yes	102.02	6	1
2j	Bicyclogermacrene	204.35	0.00	3.34	0	0	Yes	109	0	1
2k	Castilliferol	432.38	137.43	2.67	4	8	Yes	61.58	5	0
2l	Patuletin	332.6	140.59	2.02	5	8	Yes	60.49	2	0
2m	Quetcetin	302.24	131.36	1.63	5	7	Yes	63.68	1	0

Comp Code	Ligand	Physicochemical Properties					Lipinski's Rule	%Abs[d]	N viol[e]	N rot[f]
		MW[a]	TPSA[b]	logP[c]	H_A	H_D				
2n	Epicatechin	290.27	110.38	1.47	5	6	Yes	70.91	1	0
2o	Germacrene B	204.35	0.00	3.27	0	0	Yes	109	0	1
2p	Chlorogenic acid	354.31	164.75	0.96	6	9	Yes	52.16	5	1
2q	Stigmasterol	412.69	20.23	5.01	1	1	Yes	102.02	5	1
2r	Pomolic acid	472.70	77.76	3.60	3	4	Yes	82.17	1	1
2s	α -Humulene	204.35	0.00	3.27	0	0	Yes	109	0	1
2t	Omeprazole	345.42	96.31	1.64	5	1	Yes	75.88	0	5

[a]MW: molecular weight, [b]TPSA: total polar surface area, [c]miLogP: molinspiration partition coefficient, [d]%Abs: %Abs = 109 – (0.345 × TPSA),[e]nviol: number of violations,/nrotb: number of rotatable bonds, H_A: number of hydrogen bond acceptors, H_D: number of hydrogen bond donors

Table 5: Toxicity Profile of constituents of *Centella asiatica*

Ligand	Acute Toxicity	Carcinogenicity (Mouse)	Mutagenicity	Carcinogenicity (Mouse TD50)
Asiaticoside B	Not Probable	0	0	0
Madecassic acid	Not Probable	0	0	0
Brahminoside	Not Probable	0	0	0
Castillicetin	Highly Probable	0.217	0.418	95
Centellasaponin B	Not Probable	0	0.0598	0
Centellasaponin C	Not Probable	0	0.063	0
Asiaticoside E	Not Probable	0	0.0683	0
Asiaticoside F	Not Probable	0	0	0
Asiaticoside G	Not Probable	0	0.0686	0
Omeprazole	Not Probable	0	0.18	0

favorable ADME and toxicity characteristics, they can all be considered plausible lead candidates.

Discussion

An issue with world health is peptic ulcer disease (PUD). With higher recurrence rates, its etiology is complex and multifactorial. To stop the recurrence of stomach ulceration, hyperacidity, and other conditions, novel methods are required. The purpose of the current study is to examine the antiulcer activity of constituents of *Centella asiatica* in peptic ulcer disease. This research study's objective was to investigate the anti-ulcer potential of constituents of *Centella asiatica* by molecular docking, physicochemical properties investigation, and confirmation of its pharmacological effects at the molecular level utilizing various molecular techniques.

The best binding affinity Kcal/mol were predicted for *Centella asiatica* constituents are Asiaticoside B (-17 Kcal/mol), Madecassic acid (-14.6 Kcal/mol), Brahminoside B (-10.4 Kcal/mol), Castillicetin (-10.3 Kcal/mol), Centellosaponin B (-10.3 Kcal/mol), Centellosaponin C (-10.0 Kcal/mol), Asiaticoside E (-9.9 Kcal/mol), Asiaticoside F (-9.9 Kcal/mol), Asiaticoside G (-9.9 Kcal/mol), Centelloside E (-9.9 Kcal/mol) and for standard drug to be Omeprazole (-7.3 Kcal/mol).

The bioactivity score offers details on the drug binding cascade that is utilised to create a new functional medicine using constituents with a higher binding selectivity profile and fewer side effects. All chosen anti-ulcer constituents underwent toxicity profile evaluation and are included in Table 5. Except for Castillicetin, all of

the constituents were determined to be unlikely to cause toxicity.

Molecular docking is an analytical technique with a principle involving the binding mechanism of a ligand with a target protein having a known 3D structure. It is an essential tool in structure-based computer-aided drug design. The suggested *Centella asiatica* constituents are effectively docked into the target protein's active site using PyRx software. With the target protein (PDB ID: 2XZB) some constituents like Asiaticoside B, Madecassic acid, and Brahminoside form effective hydrogen bonds. The most effective binding between constituents of *Centella asiatica* and protein was compared with that of the binding of standard compound (omeprazole) and the target protein effectively by docking experiments.

Designing novel pharmacological substances requires an understanding of physicochemical characteristics. These molecule-specific characteristics, such as molecular weight, HBA, HBD, rotatable bonds, TPSA, number of heavy atoms, and molar refractivity, can be used to assess the drug similarity profile. For the substances listed in Table 4 of Asiaticoside B, these parameters were calculated.

Docking scores of Asiaticoside B are better than Omeprazole, predicting that Asiaticoside B will be more active and effective in terms of binding. In post-docking analysis, it was observed that compoundsAsiaticoside B, Madecassic acid, Brahminoside, Castillicetin, Centellasaponin B, and Centellasaponin C are binding to the active site in almost at the exact spot as Omeprazole. According to the docking score and binding poses of constituents like Asiaticoside B, Madecassic acid, and Brahminoside, it is evident that they are energetically better than Omeprazole as shown in Figure 3.

Absorption, penetration via the skin, distribution, metabolism/biotransformation, and excretion were among the expected pharmacokinetic parameters. It is well established that the intestinal estimated permeation method (BOILED-Egg) may accurately anticipate results for intestinal absorption and passive cerebral infusion. The white part of this predicted model reflects passive stomach absorption, whereas the yellow section shows passive cerebral permeability for molecules, respectively.

The BOILED-EGG curve obtained bro the SWISS ADME prediction tool is displayed in Figure 5. This approach allows for the prediction of the drug's GI absorption, biological membrane penetration, and BBB penetration. The prediction consisted of Asiaticoside B in the GI absorption zone (HIA) and not in the BBB penetration zone (yolk). Any component identified in the gray zone is indicative of absent GI absorption or BBB penetration. Thus Asiaticoside B consists of required biological membrane penetrability. SWISS ADME predictions also demonstrated that these constituents do not act as P-gp substrates as it is not susceptible to the P-gp efflux mechanism. This P-gp efflux mechanism is said to be exploited by many cancer cell lines to build drug resistance which is thereby understood to be absent in Asiaticoside B (Table 4).

As shown in Table 3, Asiaticoside B has minimal GI absorption and no BBB penetration, which is clear from this prediction finding. A medicine that can be administered orally or transdermally can be predicted and identified using the skin permeability model. According to the model, a molecule is said to have less skin permeability if its log Kp value is more negative. Asiaticoside B was determined to have the lowest level of skin permeability.

Since cytochrome P450 enzymes play an important role in drug removal through metabolic transformation, interaction between molecules and these isoenzymes is crucial. By decreasing the solubility and causing the drug or its byproducts to accumulate, inhibition of these isoenzymes may have unintended undesirable side effects. CYP2C9, CYP2D6, CYP1A2, and CYP3A4 are not inhibited by the substance Asiaticoside B, according to prediction. Asiaticoside B has no inhibitory effect on the isoenzyme CYP2C19 molecule.

Conclusion

The *in silico* characteristics of *Centella asiatica* constituents were investigated. According to the ADME study, all produced compounds can be regarded as lead molecules. All constituents were investigated using molecular docking and virtual screening. The docking score for compound Asiaticoside B is -17 Kcal/mol whereas the common medication Omeprazole was evaluated to be -7.2 Kcal/mol. More binding affinity is indicated by a higher negative score, which also indicates the most potent inhibitor.

Along with docking studies, ADME analysis assisted in obtaining the property of constituents of *Centella asiatica*. Swiss ADMET predicted biological membrane penetration, physic-chemical properties along toxicology hints. This prediction showed that these constituents can be absorbed efficiently through the skin and digestive systems but do not penetrate BBB. Further pharmaco-kinetic properties by these analysespredict that these constituents of *Centella asiatica* are nonsubstrates of P-gp and have non-toxicological behaviour.

The last step of HCl production involves the working of a proton pump in parietal cells, where the exchange of H^+ and K^+ occurs. Thereby proton pump inhibition will lead to reduced acid secretion, improved mucous secretion and protection along with better and rapid ulcer healing effects.

Therefore, these constituents indicated that they provide better proton pump inhibitory action in comparison to the common medication Omeprazole, which is an irreversible proton pump inhibitor. As in comparison with Omeprazole these constituents are predicted to produce minimal adverse effects as they are plant-based natural products.

Ethical Statement

In this context, an ethical statement does not apply, as no formal ethical requirements or guidelines are imposed or expected.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. They collaborated in designing the research, conducting thorough analysis, drafting the manuscript, and refining its content. Their collective efforts ensured a well-structured and high-quality study, reflecting their shared commitment to its successful completion. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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