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EUPATORIUM REBAUDIANUM BERTONI (STEVIA): INVESTIGATING ITS ANTI-INFLAMMATORY POTENTIAL VIA CYCLOOXYGENASE AND LIPOOXYGENASE ENZYME INHIBITION - A COMPREHENSIVE MOLECULAR DOCKING AND ADMET ANALYSIS

Dr. Bikramaditya Mukherjee [1], Dr. Garima Bartariya [2], Habibullah Khalilullah [3], Balan P. [4], Dr. Revan Karodi [5], Aditya Vikram Jain [6], Dr. Priyanka Kamaria [7]* and Suraj Mandal [8]

[1] Assistant Professor, Department of Biochemistry, Manipal Tata Medical College, Jamshedpur, Manipal Academy of Higher Education, Manipal, India.
[2] Associate Professor, IIMT University, O-Pocket, Ganganagar, Meerut, U.P., India.
[3] Assistant Professor, Department of Pharmaceutical Chemistry & Pharmacognosy, Unaizah College of Pharmacy, Qassim University, Saudi Arabia.
[4] Professor & Head, the Erode College of Pharmacy, Veppampalayam, Vallipurathanpalayam (PO), Erode, Tamilnadu, India.
[5] Associate Professor, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune.
[6] Assistant Professor, College of Pharmacy, Teerthanker Mahaveer University, Delhi Road, Moradabad.
[7] Assistant Professor, KLE College of Pharmacy, Bangalore Affiliated to KLE Academy of Higher Education and Research, Belagavi, Karnataka (* Corresponding Author)
[8] Assistant Professor, Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, O-Pocket, Ganganagar, Meerut, U.P., India.

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Abstract

Using molecular docking and ADMET analysis, this study provides a comprehensive investigation into the anti-inflammatory properties of Eupatorium Rebaudianum Bertoni (Stevia), focusing on its interactions with the enzyme's lipoxygenase and cyclooxygenase. We shed light on the Stevia compounds' binding affinities with these enzymes through in silico approaches, providing an understanding of their inhibitory effects. One herb that has been used extensively to relieve irritation is Stevia rebaudiana Bert. Numerous articles have presented rational evidence supporting Stevia rebaudiana Bert's anti-inflammatory properties. Regardless, there has never been any research on the bioactive compound that contributes to its anti-inflammatory effects. The purpose of this study was to identify the particles that control Stevia rebaudiana Bert's anti-inflammatory properties while also investigating their pharmacokinetics and toxicological characteristics. ADT resource to COX-1, COX-2, and 5-LOX aided the molecular docking screening of the strong phytochemical acquired from Stevia rebaudiana Bert. Additionally, the ADMET projection demonstrated the remarkable pharmacokinetic and toxicity profile of such bioactives. Thus, it is possible to think of stigmasterol, campesterol, and β-sitosterol as evolved anti-irritation experts. In any event, it is crucial to carry out additional research on their anti-inflammatory routines while keeping in mind both in vitro and in vivo testing.

Keywords: Eupatorium Rebaudianum Bertoni, Stevia, Anti-Inflammatory, Potential Via, Cyclooxygenase, Lipoxygenase, Enzyme Inhibition, Molecular Docking, ADMET Analysis.

1. INTRODUCTION

Rebaudianum Eupatorium Bertoni, also referred to as stevia, is a native plant of South America, particularly Paraguay and Brazil, where it has long been used as a distinctive sugar and healing spice. A group of compounds known as steviol glycosides are found in its leaves, with stevioside and rebaudioside A being the most abundant and significant components. These blends are extremely sweet, containing far fewer calories than sugar, making stevia a popular choice for replacing sugar in a variety of

food and beverage products. Beyond its enhancing qualities, more research has provided light on possible medical benefits, particularly in the area of anti-inflammatory action.

Numerous chronic ailments, such as diseases, heart attacks, and neurological disorders, are linked to the inflammatory cycle. Enzymes, for example, lipoxygenase (LOX) and cyclooxygenase (COX) assume significant parts in the creation of favourable to inflammatory arbiters like prostaglandins and leukotrienes in moderately aged people. By inhibiting these enzymes, associated diseases may be alleviated and the inflammatory response modified. Due to its anti-inflammatory effects and growing revenue in regular builds, stevia has emerged as a good candidate for further research.

A computational technique called molecular docking looks at simulating the molecular level of contact between target proteins and small particles. Analysts can predict the binding preference and mode of interaction between bioactive combinations in stevia and important enzymes involved in the inflammatory cascade, such as COX and LOX, by using molecular docking experiments. This method provides important tidbits of information about possible systems that Stevia's anti-inflammatory effects may be hidden from.

Furthermore, pharmacokinetic and toxicological characteristics of bioactive combinations are important factors to consider while developing new drugs. A logical assessment of these cut off points is given by the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis, which works with the distinguishing proof of lead compounds with great pharmacological profiles and insignificant unfriendly impacts. A thorough understanding of the pharmacological potential and security profile of Stevia-inferred compounds as anti-inflammatory specialists is provided by combining ADMET analysis with molecular docking investigations.

We hope to examine the anti-inflammatory properties of stevia in this review by employing a comprehensive molecular docking study with an emphasis on the COX and LOX enzymes. Additionally, we will oversee ADMET study to assess the security profile and pharmacokinetic characteristics of identified bioactive combinations. This study aims to expand routine choices for inflammatory conditions and prepare for future helpful interventions by elucidating the molecular components fundamental to Stevia's anti-inflammatory effects.

2. LITERATURE REVIEW

Atas et al. (2018) Investigated on how nitrogen doses affect stevia's antibacterial and antioxidant properties. Several nitrogen manure dosages applied to Stevia plants were examined, and the resulting variations in antioxidant and antibacterial qualities were noted. The study found that the antioxidant limit of stevia was affected by varying nitrogen doses, suggesting that nitrogen may have a role in controlling the antioxidant activity of the plant. Additionally, changes in antimicrobial mobility were observed, suggesting a relationship between nitrogen treatment and the plant's defense mechanisms against bacteria.

Bender et al. (2015) investigated Stevia rebaudiana Bertoni's cellular characteristics and antioxidant exercises. Their analysis aimed to elucidate the essential elements behind stevia's protective effects as an antioxidant and its possible uses in cell security. By using a series of in vitro assays and cell experiments, Drinking Spree et

al. demonstrated significant antioxidant activity associated with Stevia extracts. The review also discussed the characteristics of the plant's cells, suggesting possible benefits in protecting against cell damage caused by oxidative pressure.

Covarrubias-Cardenas et al. (2018) inspected the phenolic profile and antioxidant breaking point of dry powder concentrates of Stevia rebaudiana got with ultrasonic help. The experts portrayed the phenolic forces found in Stevia removes utilizing progressed logical strategies, for example, ultra-execution liquid chromatography combined with photodiode bunch ID and electrospray ionization mass spectrometry. The survey uncovered an unmistakable phenolic profile with major areas of strength for a breaking point, underscoring the potential advantages of phenolics got from stevia for further developing prosperity.

Dkhil et al. (2020) inspected the antibacterial and anthelmintic properties of leaf removes from Indigofera oblongifolia. They anticipated that their survey would assess this plant species' conceivable application in conventional medication and country rehearses. Dkhil et al. exhibited solid anthelmintic impact against parasitic worms and antibacterial activity against different microbiological microorganisms through a progression of in vitro and in vivo tries. These discoveries recommend that Indigofera oblongifolia can possibly be an unmistakable solution for helminth contaminations and microbiological sicknesses.

Ferrazzano et al. (2015) commissioned an investigation to assess if Stevia rebaudiana Bertoni qualifies as a non-cariogenic sweetener. Their review organized previous research on the effects of stevia on dental health and the development of caries. Ferrazzano et al. concluded from a thorough review of both in vitro and in vivo tests that Stevia has very little cariogenic potential when compared to sucrose and other artificial sweeteners. According to the poll, Stevia might be a safer alternative for those wishing to cut back on sugar without jeopardizing their tooth health.

Gawel-Beben et al. (2015) investigated the Stevia rebaudiana Removes from Bertoni leaves are a multipurpose source of regular antioxidants. Their review aimed to illustrate the anti-oxidant qualities of stevia extracts and explore their possible uses in food and pharmaceutical industries. Through a series of biochemical experiments and spectroscopic studies, Gawel-Beben et al. demonstrated the vital antioxidant activity associated with extracts from Stevia leaves. The review also highlighted the existence of several bioactive mixes, such as flavonoids and phenolic compounds, that may have positive effects on wellbeing.

3. MATERIALS AND METHODS

3.1. Research Tools and Materials

The apparatuses utilized in this examination were modifying and devices. A HP DESKTOPUFDJ08O PC with AMD E2-9000e RADEON R2, hammer 4 GB, and hard circle 500 GB is the device utilized. The accompanying items are utilized: Microsoft Succeed 2010, Chem Draw Ultra 12.0, Chem3D Star 12.0, BIOVIA Divulgence Studio Visualizer, Autodock4, Notepad+++, and Windows 10 Pro working system. The SCF Bio site is utilized in Lipinski's Norm of Five Screening Technique. ADMET expectations made with the PK CSM site.

COX-1 enzymes with PDB id 1EQG, COX-2 enzymes with PDB id 6COX, and 5-LOX enzymes with PDB id 6NCF were the materials utilized in the audit. Stevia rebaudiana Bert, a plant substance, gave the ligand's plan.

3.2. Prediction of Physioco chemical Properties

This study's conjecture of physicochemical characteristics was done involving Lipinski's Norm of Five technique related to the SCF Bio site. Molecular Weight, Log P, Hydrogen Bond Donors (HBD), Acceptors (HBA), and Molar Reactivity are the limits. The parts that are currently available through the SCF Bio site are coordinated with the state of the art 2D construction. Table 1 that goes with it presents the impacts of physicochemical property assumptions.

Table 1: Value of the Validation Results' RMSD

PDB Code	RMSD Value (Å)
1EQG	1.955
6COX	1.780
6NCF	2.738

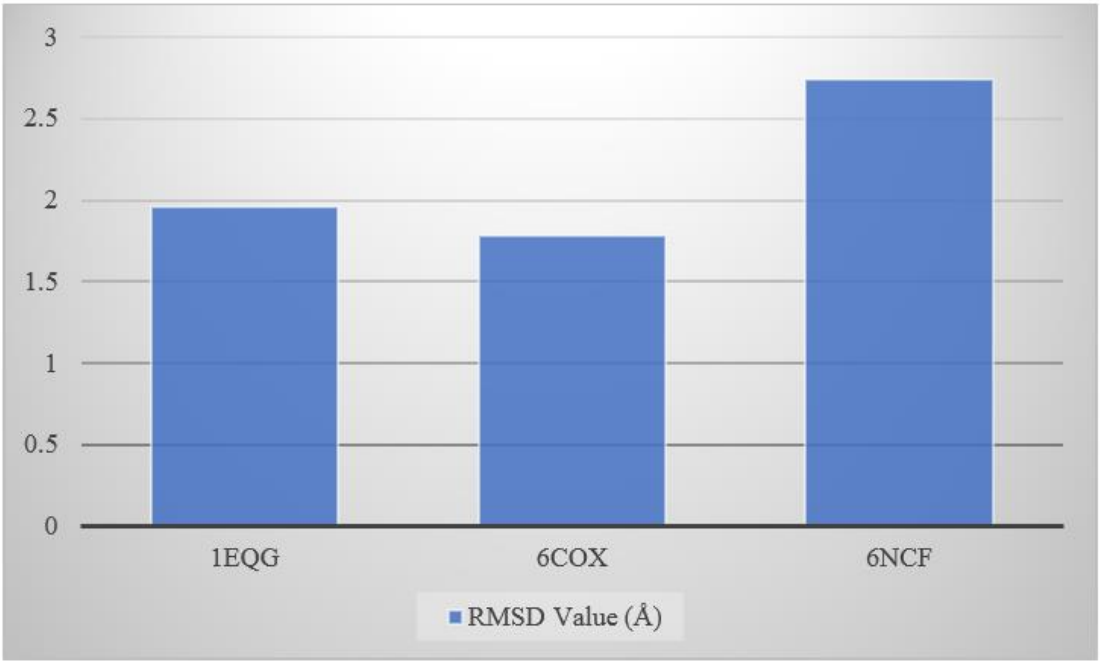

Figure 1: Graphical Representation of Value of the Validation Results' RMSD

3.3. Prediction ADMET

To examine the pharmacokinetic attributes of the spice Stevia rebaudiana Bert, the absorption, distribution, metabolism, removal, and toxicology (ADMET) assumptions were completed. ADMET expectation utilizing each test compound's Smiles code and the pk CSM.

3.4. Ligan Preparation

Rebaudiana Stevia Bert. plant test turns out to be more serious This audit included fourteen blends. Licofelone is the assessment substance that is utilized. These blends are addressed in two aspects and afterward changed over into three aspects molecular models utilizing the Chem3D Expert 12.0 program.

3.5. Protein Preparation

Enzymes cloclooxygenase-1 (PDBid code: 1EQG), COX-2 (PDBid: 6COX), and 5-LOX (PDBid: 6NCF). Utilizing the BIOVIA Exposure Studio Visualizer application, protein structures are isolated from solvents, nearby ligands, or fabricate ups by tapping the record, opening, and looking for the separated receptor report envelope, which causes the protein design to show up in a three-layered model. Then, select water/ligands/protein chains, click on the eliminate choice, and save the receptor report in.pdb configuration design.

Utilizing BIOVIA Disclosure Studio Visualizer, neighbourhood ligands from the protein structure are removed for receptor endorsement by tapping the record, opening it, and finding the envelope containing the receptor report. To eliminate solvents and proteins (amino acids), click the water or protein chains button on the control center, then select Dispense with or Press Delete. Proteins liberated from nearby ligands and unnecessary aggregations are ready.

3.6. Methode Validation

To characterize the system box, open the coordinated 1EQG, 6COX, 6NCF, and neighborhood ligand enzymes on the Auto Dock instruments worksheet. There are moves toward taking care of the grid. Click the system, trailed by the organization box, center, lastly, ligand-centered center. To save the organization choice, select Record and afterward Close Saving Current. To save the organization box limit report, tap the cross section, select Outcome, and afterward select Save GPF.

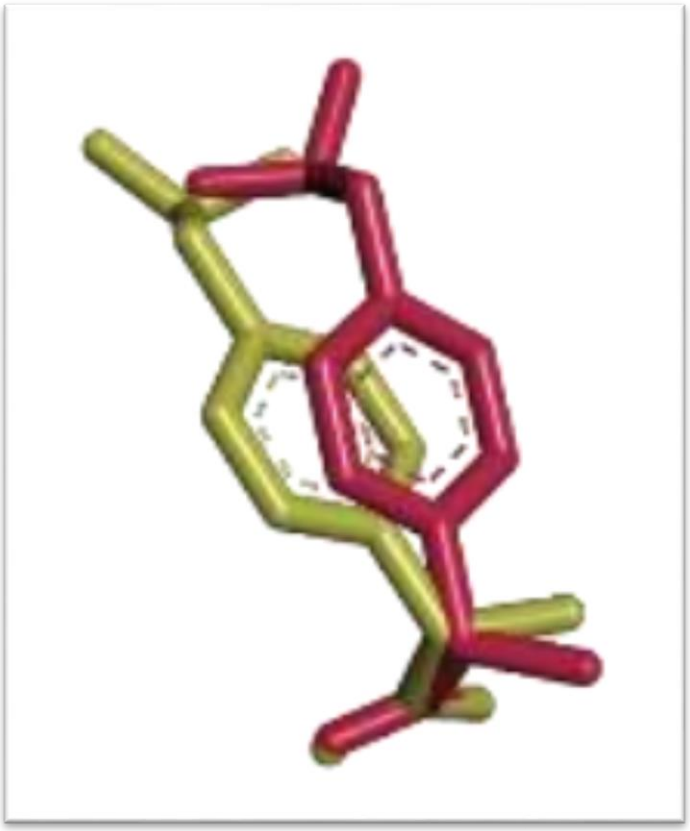

Figure 2: Visualizations that Overlap 1EQG's Native (pink) and re-docking (yellow) Ligands have an RMSD Value of 0.955 Å

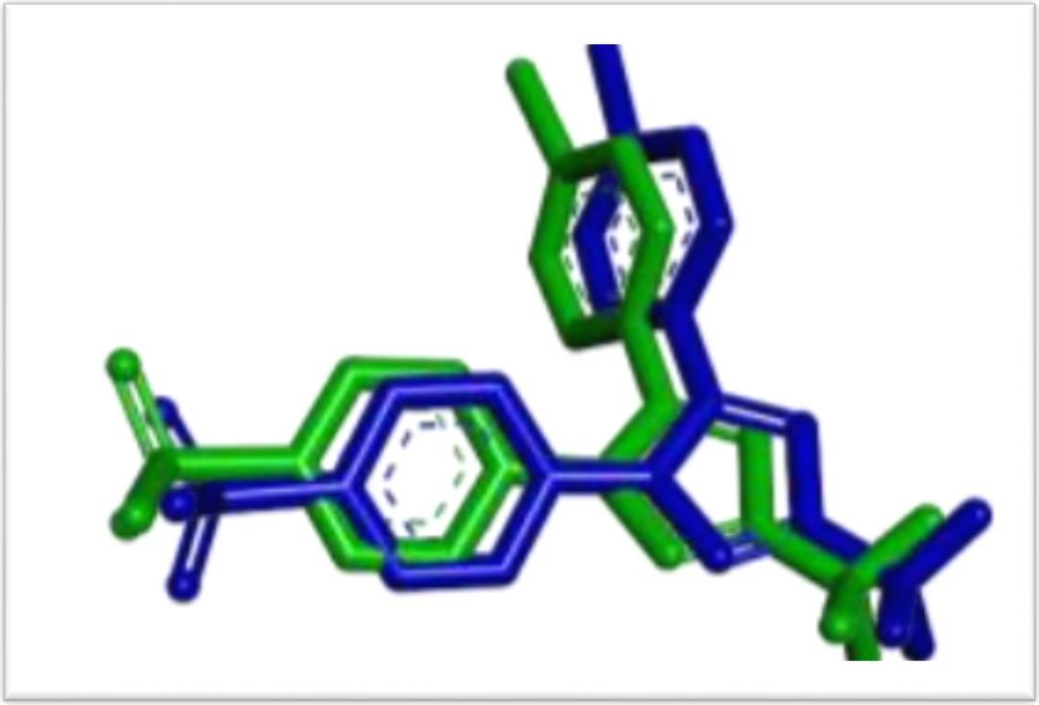

Figure 3: Visualization of the overlapping 6COX native ligand (blue) and re-docking ligand (green) with an RMSD value of 0,780 Å

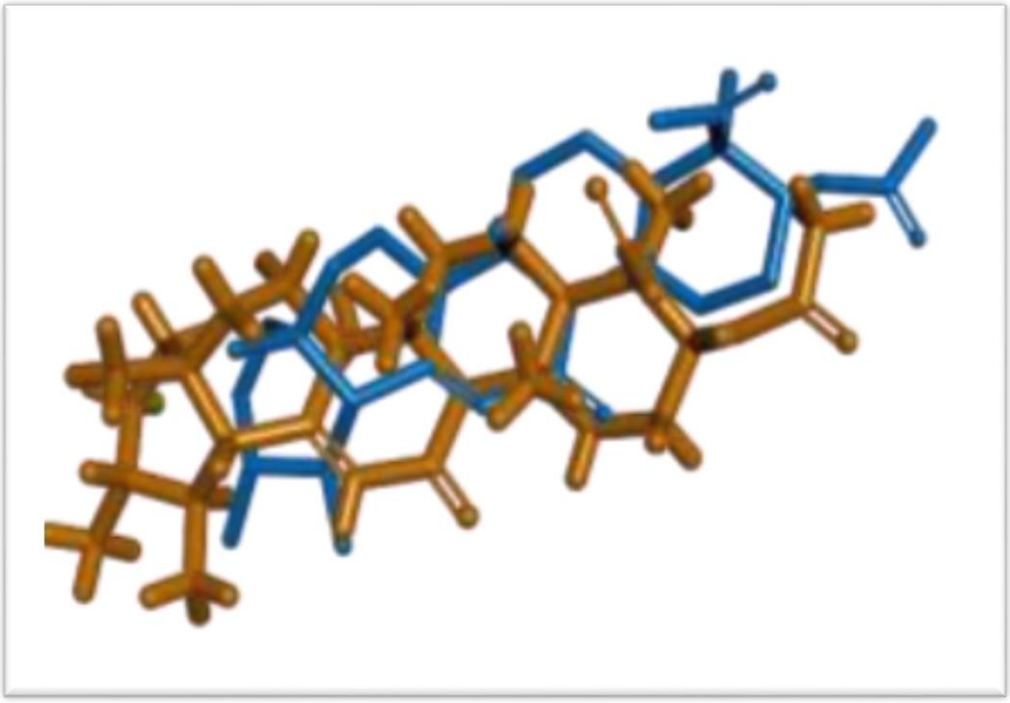

Figure 4: Visualizations that overlap the orange native ligand of 6NCF and the light blue re-docking ligand have an RMSD value of 1,738 Å

The docking limits are laid out through the Innate Estimation; the boundaries for different limits are laid out normally, and the quantity of GA runs is duplicated by the ligand receptor affiliation. Docking in the test builds the differences in the section of the grid limits that are adjusted in consistence with the procedure's endorsement.

Table 2: Forecasting Physicochemical Properties

No.	Test Compounds	Molecular Weight (BM)	Log P	HBA	HBD	Molar Refractivity
1	Austroinulin	324	4.46	4	4	96
2	b-sitostesol	416	9.04	2	2	129.24
3	campesterol	402	8.65	2	2	124.62
4	dulkosida-A	790	-2.93	18	11	185.98
5	Rebaudiosida-A	968	-6.13	24	15	220.14
6	Rebaudiosida-B	806	-3.39	19	12	187.92
7	Rebaudiosida-C	952	-5.10	23	14	218.63
8	Rebaudiosida-D	1130	-8.30	29	18	252.67
9	Rebaudiosida-E	968	-6.12	24	15	220.02
10	Rebaudiosida-F	938	-5.48	23	14	214.03
11	Sterebin A	312	3.09	5	4	86.19
12	Steviolbiosida	644	-1.22	14	9	155.18
13	Steviosida	806	-3.96	19	12	187.41
14	Stigmasterol	414	8.82	2	2	129.13

4. RESULTS AND DISCUSSION

4.1. Validation of Methods or Redocking

Redocking the nearby ligand on the three enzymes — explicitly, 1EQG, 6COX, and 6NCF — that were recovered from the Protein Data Bank site finished the procedure endorsement for this examination.

The endorsement results that should be investigated include taking a gander at every enzyme's RMSD esteem. Molecular docking approaches use RMSD values < 2.0 Å as progress rules.

The docking technique may be used for test intensities up to 10, as it is expected to be significant if the RMSD esteem is less than 2 Å. Considering Table 3, the three enzymes' RMSD values are less than 2.0 Å.

This indicates that the docking approach is effective, which is why it is frequently applied to the test substance.

Table 3: Stevia Rebaudiana Bert's Plant Compounds' Absorption, Distribution, and Toxicity were Predicted, and the Results Showed.

Test Compounds	Absorption		Distribution		Toxicity	
	HIA (%)	Caco-2 (10^-6 cm/s)	PPB (%)	BBB	Hepatotoxicity	AMES Test
Austroinulin	95.227	2.45	85.440	1.349	No	No
b-sitosterol	95.465	2.202	32.398	1.782	No	No
campesterol	95.544	2.224	99.677	1.775	No	No
dulkosida-A	8.92	-1.757	86.626	-2.544	No	No
Rebaudiosida-A	1	-2.392	99.672	-3.617	No	No
Rebaudiosida-B	1	-1.858	99.315	-3.138	No	No
Rebaudiosida-C	1	-2.137	42.587	-3.193	No	No
Rebaudiosida-D	1	-2.735	29.647	-4.247	No	No
Rebaudiosida-E	1	-2.44	24.992	-3.660	No	No
Rebaudiosida-F	1	-2.377	30.216	-3.449	No	No
Sterebin A	97.025	1.868	27.272	-1.709	No	No
Steviolbiosida	2.75	-1.689	20.553	-2.56	No	No
Steviosida	1	-2.088	24.947	-3.030	No	No
Stigmasterol	95.98	2.214	25.859	1.772	No	No

4.2. The Rule of Five Predictions by Lipinski

The physicochemical properties of a compound are examined utilizing Lipinski's Norm of Five assumptions, which comprise of five limits: the molecular heap of the compound ≤ 500 Da; the (HBD) addressed by the quantity of O-H and N-H gatherings ≤ 5; the (HBA) addressed by the quantity of O and N particles ≤ 10; the logarithmic worth of the octanol section coefficient (Log P) ≤ 5; and the molar refractivity 40-130 11. Lipinski's standard ought to be met by the test substance, and a constraint of one limit ought not be met. There are only two blends — austroinulin and sterebin A — that can be controlled orally that breeze through Lipinski's standard assessment.

4.3. ADMET Prediction

Drug plan frustration rates and gathering framework costs are the reason for ADMET's assessments. ADMET hopes to foresee a compound's pharmacokinetic and toxicity qualities in light of its molecular design. estimates from ADMET utilizing the pk CSM Online Gadget site.

The limits that were analyzed incorporated those connected with absorption (Human Intestinal Absorption; HIA) and Caco-2 cells; distribution (BBB); metabolism (CYP inhibitors); excretion (CYP2C19, CYP2C9, CYP2D6, and CYP3A4); mutagenicity (Ames Toxicity); and reactions connected with liver harm (hepatoxicity).

By looking at the inhibitory power of these blends against cytochrome enzymes, the metabolic profile was not totally settled. The CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 are the five principal isoforms.

On account of diminished opportunity and medicine assortment, inhibition of these five isoenzymes may bring about drug associations connected with pharmacokinetics that actuate unfriendly secondary effects or undesired prescription reactions.

One significant isoform of the cytochrome P450 enzyme is subdued by austroinulin, β-sitosterol, campestrol, and stigmasterol synthetic substances, as per ADMET anticipated results. test combinations docked against 5-LOX, COX-2, and COX-1 enzymes.

Table 4: Findings from Ligand Docking with COX-1, COX-2, and 5-LOX Enzymes

Test Compounds	Docking Score		
	COX-1	COX-2	5-LOX
Austroinulin	-9.19	-9.24	-7.38
β-sitosterol	-12.13	-12.46	-6.96
Campesterol	-12.44	-12.35	-12.35
Dulkosida-A	+35.16	+34.03	-8.31
Rebaudiosida-A	+84.32	+150.85	-5.42
Rebaudiosida-B	+34.49	+52.38	-6.96
Rebaudiosida-C	+105.45	+123.93	-5.43
Rebaudiosida-D	+435.30	+515.91	-1.89
Rebaudiosida-E	+96.93	+183.45	-5.18
Rebaudiosida-F	+91.12	+84.48	-6.72
Sterebin A	-9.41	-8.90	-7.36
Steviolbiosida	+6.83	-1.73	-7.98
Steviosida	+32.64	+24.06	-7.62
Stigmasterol	-11.63	-12.85	-8.09
Licofelone	-8.89	-11.05	-7.93
Native Ligand	-9.56	-11.73	-11.66

4.4. Testing Compound Docking with COX-1, COX-2, and 5-LOX Enzymes

The PDB codes 1EQG, 6COX, and 6NCF relate to the COX-1, COX-2, and 5-LOX enzymes utilized in this examination. The preparation of test ligands from Stevia rebaudiana plant blends and connection ligands — all the more explicitly, licofe lone — utilizing Chem3D Master 12.0 is the most important phase in the docking framework. Following that, the set-up ligands are docked on approved proteins. The previous table ought to show the docking eventual outcomes of the test ligands, connection ligands, and nearby ligands.

Table 5: The worth of the inhibition consistent for the enzymes COX-1, COX-2, and 5-LOX

No.	Test Compounds	Inhibition Constant (IC)		
		COX-1	COX-2	5-LOX
1.	Austroinulin	2.01 uM	927.01 nM	22.60 nM
2.	β-sitostesol	8.12 nM	5.02 nM	44.75 uM
3.	Campesterol	5.22 nM	5.86 nM	5.86 nM
4.	Dulkosida-A	-	-	5.47 uM
5.	Rebaudiosida-A	-	-	586.78 uM
6.	Rebaudiosida-B	-	-	44.75 uM
7.	Rebaudiosida-C	-	-	574.91 uM
8.	Rebaudiosida-D	-	-	226.97 uM
9.	Rebaudiosida-E	-	-	874.16 uM
10.	Rebaudiosida-F	-	-	65.76 uM
11.	Sterebin A	693.65 nM	2.66 uM	23.13 uM
12.	Steviolbiosida	-	295.54 mM	8.50 uM
13.	Steviosida	-	-	15.19 uM
14.	Stigmasterol	17.44 nM	3.11 nM	223.06 nM
15.	Licofelone	2.68 uM	44.49 nM	9.47 uM
16.	Native Ligand	542.14 nM	14.88 nM	16.75 nM

5. CONCLUSION

Stevia offers examiners and farmers an extra open door similarly. It is anticipated that a huge collection of information relating to contamination control and creation strategies will improve yearly creation. The assessment of 14 Stevia rebaudiana Bert. plants considering Lipinski's Norm of Five uncovers that austroinulin and stere canister While specific blends are supposed to be less compelling when utilized as oral medications, a few combinations are supposed to have the choice to be utilized thusly. In light of binding liking values and inhibition constants, the plant Stevia rebaudiana Bert's. The two blends quickly hinder COX-1 and COX-2 enzymes in a vague way. Tyr355 and Arg120 are known as amino destructive aggregations that are engaged with ligand contacts with COX-1 enzymes. The amino acids Arg120, Phe517, Gln192, and Ser353 are known to be engaged with ligand collaborations with COX-2 enzymes. Stevia rebaudiana works preferred with 5-LOX receptors over with test blends and neighbourhood ligands, as proven by binding liking values and inhibition constants, than with the plant's campest Erol part. Since campest Erol mixtures can repress COX-1, COX-2, and 5-LOX enzymes, they are likewise vague.

The amino corrosive gatherings that are known to be associated with protein-ligand communications incorporate Arg101, Thr137, Arg138, Val109, and His130. In view of its pharmacokinetic attributes, the plant compound Stevia rebaudiana Bert is the campest Erol compound. Its pharmacokinetic profile is acceptable.

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